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SUPPLEMENTUM 430

ALBUMIN

Investigations into the metabolism distri
transfer of albumin under normal and certain
pathological conditions with special reference to
the gastro-intestinal tract

A clinical and experimental study

By

JARL WETTERFORS

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STOCKHOLM 1965

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Investigations into the metabolism, distribution, and transfer of albumin under normal and certain pathological conditions, with special reference to the gastro-intestinal tract.

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BOK OCH REKLAMTRYCK AB

To My Wife

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II. *The ormal passag f erum-albmin into the gastro-intestinal tract nd its role in th catabolism f albumin.* An experimental study in the dog. Acta Med. Scand. 176 787 1964

III. *Catabolism and distrib tion of serum alb min in the dog* Acta Med. Scand. 177 243 1965.

IV *Catabolism nd distribution of alb m in gastric an er* Together with Liljedahl, S-O Plantin, L.-O and Birke, G Acta Med. Scand. 172 163 1962.

V *Hypoalb minaemia in ulcerative colitis nd ertain forms f ent ritis.* Together with Liljedahl, S.-O Plantin, L.-O and Birke, G Acta Med. Scand. 174 529 1963

VI *The acut radiation synd ome the mportance f th gastro-int stinal injury in the atabolism and distribution f serum-albumin.* Together with Liljedahl, S.-O Plantin, L.-O and Birke, G Acta Med. Scand. 177 227 1965

Part I

INTRODUCTION

Albumin is the main component of the serum proteins of which it constitutes 55—70 %. With a molecular weight of 69,000 it stands for about 80 % of the colloidosmotic pressure of the blood. It serves as carrier for a lot of substances, such as hormones, bilirubin, free fatty acids, and certain metabolites (13 14). As for the possibility of its availability as a reserve protein to the cells, there are different opinions (53 59 75 177).

In many pathological conditions of surgical as well as medical interest serum albumin is subject to more or less profound quantitative changes, the nature and genesis of which are obscure.

Since the introduction of isotopes in medical work the possibility of labelling proteins with different isotopes has facilitated more dynamic approach to the problems of protein metabolism than the electrophoresis technique alone could supply.

Endogenous labelling with ^{14}C or ^{35}S is the procedure closest to nature no denaturation of the protein occurs, but there is one main disadvantage the re-utilization phenomenon. This means that to a certain extent the label of the catabolized protein molecules is utilized in the synthesis of new protein molecules, serum proteins as well as intracellular proteins (62, 102, 108, 127 169).

Furthermore, the endogenous labelling of proteins is very expensive and time consuming and the gain of labelled protein is only few per cent of the label administered. This method is thus not applicable to clinical practice.

The exogenous labelling with ^{131}I is not charged with any re-utilization of the label (55) but there is a risk of protein denaturation by too heavy iodination. Some earlier authors have doubted the possibilities of using ^{131}I-proteins for metabolic purposes (5 6, 18, 168).

However *McFarlane* (110 112) has succeeded in labelling albumin and other proteins with ^{131}I without denaturation. This fact was well demonstrated by *Campbell, Cuthbertson Matthews & McFarlane* (28) as they obtained similar biological half lives for their I and ^{14}C-preparations. *Berthold & Keller* (15) and *Freeman Matthews McFarlane Berthold & Keller* (51) in analbuminaemic subjects, have shown that the behaviour of ^{131}I-labelled and native albumin was identical.

During the last decade several papers have dealt with the problems concerning the distribution and the metabolism of ^{131}I-albumin in health and disease, and also with the theoretical-mathematical rules in the kinetics of labelled proteins. The results obtained will be reviewed in the respective chapters.

Background and aim of the investigation

The incitement to the author investigation was an observation in 1958 by *Birke Liljedahl & Plantin* (19) on severely burned patients studied by means of ^{131}I albu-

VII *Some aspects of the behaviour of serum albumin in intestinal obstruction*. An experimental and clinical study Acta Chir Scand. In press.

In the following, these papers are referred to under their Roman numerals.

Some of the investigations accounted for in this supplement have not been presented before in the above papers or elsewhere.

Lab. U.S.A. or a labelled albumin prepared at King Gustaf V Research Institute (*Plasma*) was used.

The human albumin used for labelling with ^{131}I was produced by fractionation by Cohn's method, and was obtained from AB Kabi, Stockholm.

Canine and rabbit albumin. These were prepared from homologous blood serum by electrophoresis on polyvinylchloride in veronal buffer (pH 8.6 140 mA 22 hr) (27). El tion w th physiological saline was followed by freeze-drying. The purity was checked by paper-electrophoresis and ultracentrifugation.

Labelling with ^{131}I

This was performed by McFarlane method (112)

To the protein solution buffered to pH 4–4.5 is added free iodine to oxidize the sulfhydryl groups. (At this pH the hydroxyl groups of tyrosine are not ionized.) The mixture is then passed through an anion exchange column (Zeolit FF) to remove excess of iodine and iodide. The pH of the effluent is adjusted to 9–9.5 A mixture of carrier-free ^{131}I as NaI buffered to pH 9 and iodine monochloride (I Cl) is prepared. Using a fine pipette the protein solution is then rapidly squirted into the I iodine monochloride solution. After some minutes the mixtu is passed through Sephadex-G 25 column in order to remov non-protein-bound iodine. The final product usually contains less than 1 % of non precipitable or dialyzable activity

C t ol f th l belled alb min

The human ^{131}I-labelled albumin contained less than 2 % mostly less than 1 % of non-protein-bound radioactivity

The Amersham product revealed some denaturation metabolically in the control cases, and so the results will not be accounted for. This preparation was therefore soon abandoned

In RIHSA-M and in our own preparation no significant denaturation appeared metabolically

Ultracentrifugation of native canine albumin showed an $S_{20} = 4.5$ and of the labelled product an $S_{20} = 4.6$–4.8. No components with faster sedimentation rates were revealed (fig 5 III)

By the double-diffusion technique (124) and immuno-electrophoresis (68) the immuno-characters of native and ^{131}I labelled canine albumin in the reaction with rabbit antiserum to canine serum were compared. No immunological differences were detected (figs. 1 a and b)

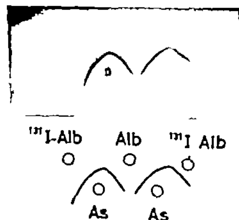


Fig. 1 a. Investigation of labelled and unlabelled canine albumin by double-diffusion technique (Ouchterlony) Iden ties of the origins are shown in the drawing. No lines indicating immunological differences are seen. ^{131}I-Alb labelled albumin. Alb nativ albumin. As rabbit antiserum.

min At autopsy a *high uptake of radioactivity* in the gastric wall was found Was this observation possibly of any significance in the problem of where serum albumin is catabolized, a problem hitherto *unsolved? The first step was to investigate* the accumulation of radioactivity in the gastric wall and the excretion of it into the stomach in unburned patients. In a case of pylorostenosis the former observa*tion was verified and a high excretion of* radioactivity found It then remained to be proved in which form the excretion of ^{131}I occurred as ^{131}I iodide solely or as ^{131}I-albumin as well Was this observation *valid in normal persons too?*

Citrin Sterling & Halsted (31) and *Gordon* (66) using ^{131}I PVP had been able to demonstrate a close connection between "exudative gastro-enteropathy" *and idiopathic hypoalbuminaemia* This connection was also verified by *Schwart & Jarnum* (141)

Was this abnormal leakage of albumin into the alimentary tract only a patholog*ical development of a physiological pro*cess? If the answers to these questions were in the affirmative, it would be readily suspected that other more or less distinctly defined pathological processes in the gastro-intestinal tract could cause protein loss, and thus, in a more specific way be responsible for the hypoalbuminaemia fairly often accompanying such conditions.

Aim

The first problem was to state qualitatively the leakage of albumin into the gastro-intestinal tract under normal conditions. The next one to perform a quantitative *study* and to correlate the figures obtained to the normal degradation of albumin. Does the leakage, provided that there are measurable quantities, play an important role in the degradation? If so do the different parts of the gastro-intestinal tract exhibit diverging properties as to the leakage?

Certain diseases in the gastro-intestinal tract of surgical as well as medical interest *are attended with hypoalbuminaemia.* Some of these diseases have been studied closely by others with I albumin and ^{131}I PVP *In the present work the interest* is focussed on some (IV V and VII) *which will be thoroughly dealt with. An* actual problem is the acute radiation syndrome in which the gastro-intestinal changes play an important role and whether these changes contribute to the *hypoalbuminaemia and the shock* (VI)

During the experimental part of the investigation certain aspects arose of the capillary permeability and the compartment analysis. These aspects were partly investigated and the results will be discussed On the basis of these investigations and of those of others into *the normal* behaviour of serum albumin some conclusions are drawn as to the existence of more general rules applying to the metabolism of albumin throughout the species *of animals (mammals)*

Own investigations

Methods

Human albumin In some of the first cases (see IV and V) and in 5 control cases I *albumin from the* Radiochemical Centre, Amersham was used. In all other cases the RIHSA M preparation of *Abbott*

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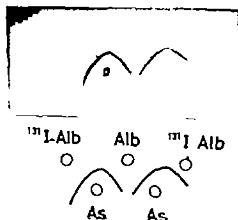


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Own investigations

Methods

Human albumin In some of the first cases (see IV and V) and in 5 control cases I-albumin from the Radiochemical Centre Amersham, was used In all other cases the RIHSA M preparation of Abbott

Determinations of radioactivity were made on 2-ml samples in a well crystal scintillation detector Tracerlab. P 20 BW connected to a Tracerlab. Superscaler SC-18 A. During the last years a Versamatic II Scaler Spectrometer was also used. The samples measured here were usually 4 ml. Correction factors were applied for the different measuring geometry in cases when smaller samples had to be used

In measurements of the samples an accuracy of ± 2 % was aimed at. In some samples of lower activity however the error of a single measurement was ± 5 %

In investigations where two isotopes were used, either ^{131}I and ^{125}I or ^{131}I and ^{51}Cr were given simultaneously measurements were made on a gamma-spectrometer (Versamatic II) in the respective gamma or X ray energy maxima, i.e. for ^{131}I 0.36 meV for ^{125}I 0.035 and for Cr 0.32 meV By measuring the standards in the respective energy ranges, correction factors for overlapping radioactivity were obtained.

Plasma- and blood-volume

Determination of the plasma-volumes was made according to the dilution principle

$$\frac{\text{Total injected dose}}{\text{activity/ml plasma}} = \text{plasma-volume.}$$

The problem of this simple method is At what time after the administration is the mixing of the label complete and how much of it has left the plasma space at that time The figures given in the literature vary between 1 and 15 minutes (37 120)

Sampling 3, 6 9 and 12 minutes after administration of the label, showed that the 3-minute sample contained activity varying greatly when compared with the following samples in all species. The samples at 6 9 and 12 minutes showed slowly falling activity

The values obtained may be treated in two ways, either by extrapolation to t = 0, and using the zero-time value for the determination, or by the use of some of the other values, preferably that of 6 or 9 minutes. It was evident that extrapolation gave values well corresponding to the 6–12-minute interval in man as well as in dogs and rabbits. *G gersen* (71) found a very small deviation of the 10-minute sample from the extrapolated zero value. *Noble & Gregersen* (120) and *v Porath* (129) stated that the error introduced when using the 10-minute value instead of the extrapolated zero value was negligible. The highest of the 6 and 9-minute values was used for the determination. In a few cases, however where there were rather great differences between the 6 and the 9 and 12-minute values in one or the other direction, the mean of the 9 and 12 minute values was used for determining the plasma-volume. A correction factor of 1.015 was used (166) to correct for the amount distributed from the plasma during the mixing time.

Recently *Huggins Smith & D avers* (81) ha e shown that in dogs the difference between the plasma-volumes obtained by using 5 or 10-minute values is insignificant. For practical purposes, either of these tw figures can be used. Here the 9-minute, or sometimes the mean of the 9 and 12-minute values, was used.

Wher they were of interest, as in IV and V the blood-volume (TBV) and red-cell olume (RCV) were calculated from

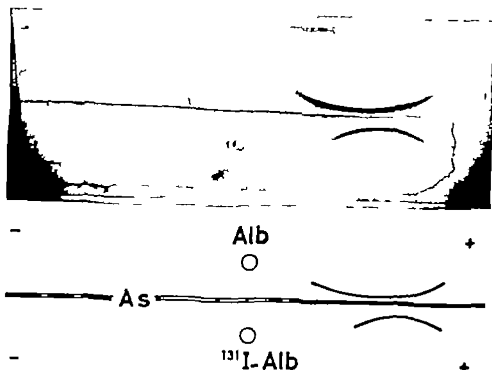


Fig. 1 b. Immunoelectrophoresis (Grabar & Williams) of labelled and unlabelled canine albumin. Identities of the origins are shown in the drawing. No immunological differences are seen, only a faster migration of the labelled albumin. Abbreviations as in fig. 1 a. (The process was carried out on microscopical slides in veronal buffer of pH 8.6, ionic strength 0.1, using the LKB 6800 A equipment.)

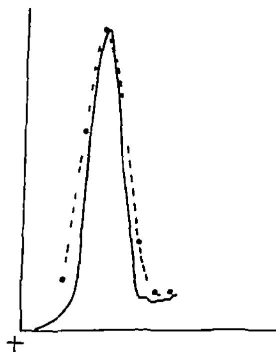


Fig. 2. Electropherogram of labelled and unlabelled rabbit albumin. There is a slight anodic dislocation of the radioactivity peak, indicating a somewhat faster migration of the ^{131}I-albumin. ●——● native albumin. ○——○ ^{131}I-albumin.

Fig. 1 b also demonstrates what is shown in fig. 2 for rabbit albumin, a somewhat faster migration velocity of the ^{131}I labelled preparation than of the native albumin.

Measurement of samples

The samples measured were plasma, gastric and intestinal perfusates, urine, faeces, and organ specimens. The faecal samples were measured after homogenization.

least possible handling of the actual segment, which was covered with a wet abdominal cloth, as the abdomen had to be kept open all the time. In dogs the non-traumatic performance is fully described in II. It was also shown in one dog (II) that ordinary manual handling of the intestine does cause injury to the mucosa, at least functionally.

The use of Thiry Vella loops in dogs was abandoned, as it has been shown by *Nylander & Olerud* (122) that the microangiographic structure changes with time. As mentioned in II, the efforts to construct isoperistaltic loops, taking part in the food passage when not investigated, were unsuccessful because of the difficulty in keeping the distal stoma tight. Special cannulas for the stomas did not keep tight or caused necrosis in the intestinal-abdominal-wall junction. The dogs did not recover fully nor gain normal appetite, and were thus metabolically unsteady.

Hitherto the peroperative perfusions are those closest to normal, as evidently anaesthesia and surgery very carefully performed did not cause any significant change in capillary transfer rate and repeated perfusions after different intervals did not alter the leakage (II). The extremely careful handling was non-traumatic and, furthermore, the slow passage of the fluid is a mechanical performance in analogy with the passage of food. Peristalsis was always present.

Ovomucoid with a molecular weight of 44,000 was used as trypsin inhibitor. In the concentration used it does not cause any hyperosmolarity. It does not change the pH as acid buffer will do, and lastly its electrophoretic behaviour is different from that of serum albumin, a fact that does not invalidate the identification of the latter. According to the manufacturer ovalbumin is as effective as commonly used soya-bean extract, that is, 1 mg inhibits 1 mg of trypsin. However, it was judged necessary to make determinations of this inhibition in vivo.

The histological examination of perfused and non-perfused intestinal segments in the experiments on dogs did not reveal any microscopical mucosal lesions or any differences between perfused and non-perfused specimens.

Methods otherwise applied in the different investigations will be discussed in the respective chapters.

Statistics

The statistical treatment of data in this publication and in the different papers is in accordance with current principles for determining means, standard deviations and standard errors of the mean, as found in most text-books of statistical methods.

In calculating regressions and correlation coefficients and in testing significances (t-tests) normal distribution is assumed.

the PV and the haematocrit value corrected for trapped plasma (0.95) and whole-body haematocrit (0 91)

Collection of gastric and intestinal samples

This part of the methods in man is well documented in paper I and in dogs in paper II but will be briefly discussed here.

In investigations on gastric excretion of albumin in man, the aim was to avoid contamination with saliva, in which a not negligible fraction of free ¹ I iodine is excreted (52) This was done either by continuous suction of the saliva or with the patient lying slightly prone on his left side instructed not to swallow but to eject salivary accumulations.

In some cases a special tube with an hour-glass-shaped inflatable balloon was *used to occlude the stomach distally In* others, suction was performed through an ordinary duodenal tube No certain loss seemed to occur in the position mentioned above.

In the first investigations in man (20) the gastric rinsing fluid was immediately withdrawn and squirted into an alkaline phosphate buffer Later on, the buffer was introduced intragastrically thus used as rinsing fluid (73)

Two points concerning the gastric sampling may be discussed The first is the *aspect of total recovery of the buffer in*troduced. At the beginning of each investigation some fluid was always lost, and the primarily introduced portions of buffer were therefore discarded. Thereafter the recovery was complete.

The use of alkaline buffer in the normochylic cases means a change of the intragastric pH which may be a possible source of error The significance of this change cannot be established as a comparison with saline perfusions with no *pepsin inhibition is not relevant. However* in two normochylic dogs gastric perfusions in both ways *were performed for* ¹¹ I albumin as well as for ¹ I iodide. No differences as to the total amount of activity excreted or to the protein bound part of it were obtained. This may indicate that the error is minimal.

Another error in the calculations is the possible attachment of iodine to macromolecules as mucoproteins or proteins of the gastric juice. This is fully evidenced in the dog and may give some overestimation of the gastric leakage, but is not of any importance in the intestinal figures *(II)*

The methods used for investigation of *the intestinal excretion of* ¹³¹I albumin are also fully described in I and II In intestinal intubations, only qualitative results are obtained. Analogous to the gastric perfusions, the buffer used here although acid may cause some error in the determinations, but probably it is very slight.

Several attempts at intestinal intubation with a double balloon tube were made in man, but even very careful *inflation of the* balloons in a normal loop under fluoroscopic control *caused peristalsis and anti*peristalsis. Thus, no constancy of the ex*perimental* conditions was achieved and moreover the patients suffered from nausea, vomiting and abdominal discomfort which necessitated the interruption of the investigation.

The peroperative perfusions in man were made extremely carefully with the

Tarver (158) *Dixon & Maurer* (39) *Yuile* et al. (178) *McFarlane* (111) *Gitlin* (56) *Matthews* (104 106) *Engström Ljunggren Persson & Wetterfors* (43) and *Friedberg* (54) The last-named author in particular presents data strongly suggesting that the process is one with constant fractional catabolic rate and that only the absolute rat (g/day) varies with the intravascular albumin content. *Reeve & Roberts* (134) are of the opposite opinion that it is a zero order process with the absolute catabolic rate being independent of the albumin available.

Freeman & Gordon (50) found that the catabolic rate decreased with decreasing albumin concentration in protein-depleted rats.

Own investigations

Studies in man

In 15 control cases, 10 investigated with RIHSA M (Abbott) and 5 with the ^{131}I albumin preparation made at King Gustaf V Research Institute *(Plasatin)* results were obtained. Some of these are accounted for in IV The two preparations agree metabolically very well with each other and therefore the results will be treated together The metabolic results are recorded in Table I In Table II they are compared with those of others.

TABLE I. *Fractional and absolute catabolic rates of albumin breakdown in 15 control cases. Values for both methods of calculation are given. M = Matthews' method and C = Campbell et al urinary clearance method. M is given only for cases investigated 12 days or more*

Subject	Intravascular albumin g/kg b.w	Degradation of albumin			
		urinary clearance (C)		graphical analysis (M)	
		% of I. pool	g/kg/day	% of I. pool	g/kg/day
1	1.56	7.8	0.12	9.5	0.15
2	1.80	9.3	0.16	10.1	0.18
3	2.40	10.2	0.24		
4	1.63	10.2	0.16		
5	1.81	8.0	0.14		
6	2.18	9.1	0.19	11.2	0.20
7	1.80	8.4	0.15	8.2	0.15
8	1.78	9.4	0.17		
9	1.52	9.9	0.15		
10	2.31	8.3	0.19	11.8	0.18
11	2.33	6.6	0.16	9.3	0.21
12	2.52	10.3	0.26		
13	2.11	9.8	0.20		
14	1.62	8.2	0.13	10.3	0.21
15	2.56	7.9	0.20	8.1	0.21
Mean ± S. E. M.	2.00 ± 0.093	8.9 ± 0.28	0.175 ± 0.01	9.8 ± 0.44	0.186 ± 0.009

Part II

ALBUMIN METABOLISM UNDER NORMAL CONDITIONS

CHAPTER I

Catabolism

General aspects

In investigations on albumin metabolism in man and in animals different methods have been employed to obtain values for the degradation. The commonest procedure has been to state the biological half life (T/2) *of the linear part of the exponential plasma activity curve. The numerical* expression for the slope multiplied by the total albumin pool, calculated by extrapolation has been used for quantitation (18 57 58, 152) This method is still in use (171)

The total albumin pool has also been used in connection with the ratio urinary *activity/retained dose for estimating the* catabolism (18 148)

However *Campbell Cuthbertson Matthews & McFarlane* (28) have pointed out the possible errors of this method as the required knowledge of the total albumin pool is seldom available. Furthermore they bring forth empirical evidence in favour of the plasma or its immediate vicinity being the catabolic site of albumin. This also gives more exact figures quantitatively as the catabolic rate is referred to a known mass of albumin Lately *McFarlane* (113) has further stressed this close correlation experimentally by using screened [131]I albumin.

Campbell et al (28) have designed the urinary clearance method now generally used in which the daily urinary excretion of activity is related to the plasma activity *The mathematical treatment of the analysis of the plasma-activity curve has been* dealt with by *Matthews* (103)

Reeve & Roberts (133) and *Reeve & Bailey* (131) have developed the actual compartment model by introducing an intermediary pool of breakdown products of the catabolized I albumin. This way of treating the model was also used by *other investigators* (11 99) A short survey of the elementary formulas devised by *Matthews* (103) is given in III

Closer knowledge of the mechanisms regulating the catabolism and its coordination with the synthesis is lacking It is known that certain hormones, namely thyroxin and corticosteroids (138) increase it but the exact mechanism is not known

The view that the breakdown of albumin is a first order process is held by

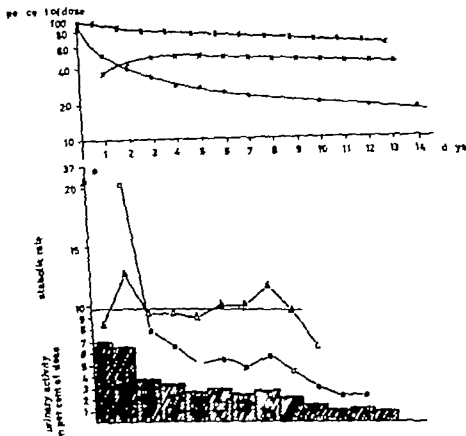


Fig. 3 Study of albumin catabolism in control case (No. 13) ■——■ retained dose ●——● plasma activity x——x extravascular activity △——△ daily catabolic rat calculated from urinary activity/intravascular activity ○——○ daily catabolic rate calculated from urinary activity/extravascular activity Hatched columns signify excreted urinary activity as percentage of dose

St dies an mals

In paper III are reported normal values for pparently healthy dogs. The mean catabolic rate is 16.9—17.7 % of the intravascular pool corresponding to 0.30—0.31 g/kg day

Data for rabbits are given in paper V An average of 21 23 % of the intravascular pool is catabolized per day that is, 0.31—0.35 g/kg/day The range is wider in this species than in man and dogs. This is probably attributable to differences between the recipients (35)

No studies on the catabolism of albumin in mice and rats have hitherto been made by the author

A short survey of the results obtained for catabolic rates, relativ and absol te is given in T ble IV (page 31) The values for r ts are taken from *Campbell* et al. (28) and *Matthews* (103) while those for

There is fairly good agreement between the two methods used (M = graphical analysis, C = urinary clearance method) Only in one case is there a marked dif ference (> 3 %) The catabolic rates (mean ± S.E.M.) are 9 8 ± 0 46 % (M) and 8 9 ± 0.28 % (C) Quantitatively this means 0 186 ± 0 009 and 0.175 ± 0 01 g/kg/day respectively

TABLE II *Comparison between catabolic rat s obtained by d fferent thors* [1]) *Calculated from intravascular albumin pool* *) *Calculated from total alb min pool.*

Authors	I travascular albumin g/kg b w	Degradation of albumin		Numbers of subjects
		% of intra vascular pool) % of total pool *)	g/kg/day	
Sterling 1951	—	6.6	0.233	21
Berson et al 1953	1 97	10.3	0.200 0.215	
Cohen & Schamroth 1958	1 36	8.8 10.2	0.125	5
Sternfeld 1960	1 40	4 6 12.6	0.180	12
	1 70	5.22 13 8	0.236	8
Jarnum & Schwartz 1960	2 12	10 0	0.206	20
		3 9		
Cohen et al. 1961	1 78	8.8 10 3	0.185	6 11
Beeken et al. 1962	1 68	4 68 ~10	0.225 0 196	13
Takeda & Reeve 1963	1 60	8.9	0.142	13
W tterfor et al. 1962 (IV)	1 95	9 0	0.17	10
Wetterfors 1964	..00	8.9 9 8	0.175 0.186	15

*) *Negroes* **) *See table I* ***) *Includes 10 cases from 1962*

The fact that there was a steady catabolic rate and no high excretion of radioactivity during the first 24 hours (2.9—7 0 %) or the next 2 days (8 2—11 5 %) indicates metabolically satisfactory preparations with apparently no protein denaturation If the urinary activity is used as numerator in a quotient with the plasma activities and the extravascular activities, respectively as de nominators, different ratios are obtained. It will be seen from fig 3 that the closest fit occurs in the former where the catabolic rate is nearly constant from day to day This contrasts greatly with the latter when reference to extravascular activity is made Here a gradual decrease is observed. This fact points to the "plasma space" as the ste of degradation. In these determina tions of degradation no intermediate pool of breakdown products was introduced.

CHAPTER II

Site of catabolism

Gitlin, Klinenberg & Hughes (58) on performing different evisceration experiments on mice, postulated that the liver and the kidneys play a significant role in the albumin catabolism. That the liver is responsible for about 10–15 % of the catabolism is indicated in perfusion experiments by *Gordon* (63), *Cohen & Gordon* (33) and *Katz, Rosenfeld & Sellers* (93). The kidneys do not participate to any significant extent in the degradation (92, 137). The reticuloendothelial system has been accredited a certain role by *Gitlin* et al. (58), while *Thorbecke, Sebestyen, Benacerraf & Green* (162) and *Freeman, Gordon & Humphrey* (49) reached quite opposing results.

That dextran molecules (m. w. ~ 78,000) are able to pass the gastric mucosa has been shown by *Troell & Åberg* (163) and *Hallgren* et al. (74). In 1959 *Birke, Liljedahl, Plantin & Wetterfors* (70) in studies with ^{131}I albumin, demonstrated the presence of albumin in gastric juice, postulated gastric catabolism of albumin, and suggested the intestine as another possible site. A autoradiographical evidence of an intestinal leakage of ^{131}I albumin was presented by *Ullberg, Birke, Ewaldson, Hansson, Liljedahl, Plantin & Wetterfors* (165). A much greater amount of radioactivity was demonstrated in the intestinal content after intravenous administration of ^{131}I-albumin than after ^{131}I iodide. *Gullberg & Olhage* (73) in 1959 showed albumin in normal gastric juice by paper and immuno-electrophoresis. *Holman, Nickel & Sleisenger* (80) showed immuno-electrophoretically its presence in intestinal juice. The same technique was used by *Barandun* et al. (9).

After this generally convincing evidence of the presence of serum albumin in the gastric and intestinal secretions, the next problem of interest was that of quantitation.

Own investigations

In paper I the results of investigations in man are reported and discussed. In II an expanded investigation into the quantitation of the gastro-intestinal leakage in dogs is accounted for. The differences in leakage between the different parts of the gastro-intestinal tract were particularly dealt with.

In I the leakage is reported as fractions of retained dose (total albumin pool) and of intravascular activity (intravascular albumin pool). The latter way of expressing the leakage must be the correct one, as the catabolism evidently occurs in the intravascular pool or close to it. To achieve a more uniform and comparable unit, independent of the actual albumin concentration, it seems better, as in II, to express the leakage in ml of plasma delivering its albumin per unit time and unit length of intestine. Such a re-evaluation gives for the stomach an average leakage

mice are recalculated from the figures of *Dixon & Weigle* (40) and *Friedberg* (54)

Discussion

It is well evidenced that [131]I delivered in the catabolism of [131]I albumin is not utilized in the resynthesis of albumin or other proteins (35 179) Thus, if denaturation can be avoided in the preparation of the labelled protein, this is an exquisite tool for the study of albumin catabolism. Screening of the labelled product in another individual of the actual species to remove occasional denaturated products, a technique used by *Lewallen Berman & Rall* (99) in man and by *McFarlane* (113) in rabbits, is of course the ideal way of achieving a metabolically pure preparation. In most cases, however this "biological screening" is impossible to carry out. That the kind of preparation used in the present investigations gave no apparent denaturation is shown by the low initial urinary excretion of radioactivity

Another requisite for making metabolic studies with [131]I albumin is that the synthesis of the endogenous substance and the administration of tracer take place in the same compartment. This has been pointed out by *Bergner* (16) in a paper on tracer dynamics in metabolic turnover studies. That albumin is synthesized in the liver and immediately released to the blood-stream has been shown by *Miller & Bale* (115) and *Jensen & Tarver* (89) For the same reason it is obligate to refer measurements of catabolism to the intravascular compartment The necessity of proving the model of *Campbell* et al. and *Matthews* to make the studies valid is clear Evidence presented up to now favours this view of intravascular degradation.

With one exception (149) there is good agreement between the results of different investigators concerning the fractional catabolic rate, calculated on the intravascular pool. Generally figures around 9–10 % are obtained in man.

Earlier investigations on animals have been performed preferably on smaller species as mice, rats, and rabbits In the two first species the total-body-counting technique has been used (40 54 58 160) *Campbell* et al. (28) however determined intravascular activity in the rat. In rabbits determinations of the catabolic rate are mostly based on intravascular activity (35 103 113 133) the results in rabbits presented above are in good agreement with these authors results.

In animals which are partially enterectomized, we do not know to what extent a compensating leakage may occur in the remaining part, as the change of blood-flow to that part has not been investigated. There is no evidence for the assumption that a partial removal of an organ may decrease its function to the same extent.

J ee bhoy & Coghill (88) and *Jeeje bhoy* (87) used ion-exchange resins orally to catch the [131]I-containing degradation products of [131] I-albumin catabolism. According to this method, about a fifth of the normal catabolism occurs by the gastro-intestinal route. *Koblet & Jeanet* (97) and *Jones & Mo gan* (91) arrived t a similar conclusion by that method. Recently this procedure has been shown to be erroneous and judged as inconvenient for quantitative conclusions (30 64)

Nor does [131]I PVP labelled according to Gordon procedure (65) seem to be a preparation suited for study of the physiological leakage of albumin. This preparation is already in vitro easily decomposed with release of the [131]iodine (83). To what extent the labelled PVP-molecule is split in the sense that [131]iodine is liberated in the small and large intestine is not known.

Another approach to the problem of quantitating the physiological gastro-intestinal leakage of albumin is that by *W aldman* (170) who used Cr-labelled albumin. H concludes that the normal leakage is a minimum. The validity of this statement will be discussed in connection with own investigations presented in the next chapter

Up to now none of the methods for determining physiological intestinal protein leakage can be shown to be more appropriate than the non-traumatic peroperative one described in II, as most of them have disadvantages which cannot be avoided.

One aspect of the perfusion technique used here, not yet covered, is that of occasional changes in the blood flow to the intestine, elicited by the perfusion. As the dogs had been starved for 24 hours prior to the investigation, the initial blood-flow to the intestine was possibly depressed. Some increase during investigations may not, therefore, be an important source of error especially as th perfusion fl id was isotonic.

Still, the quantitative roles of the bile and the pancreatic juice in albumin catabolism remain to be elucidated. However from some of the investigations in II where the papilla of Vater was not included in the perfused duodenal segment, and from 3 preliminary experiments (not accounted for here) where the common bile duct was ligated close to the duodenum, it can be stated that the influence of these secretions on the duodenal leakage per se is insignificant.

The technique used by *Campbell* et al. (29) in sheep with permanent intestinal fistulas, provided for by special cannulas, showed that the enteric juices contained a lot of albumin and the authors conclud ed that the intestine is responsible for the main part of the albumin catabolism.

The figures for the normal leakage given in I and II and their probable connection with the blood-flow to the intestine, point strongly to the gastro-intestinal tract as the main site of the normal albumin catabolism.

of 1 1 ml of plasma per hour (range 0 71 —2 2)

For the jejunum this way of calculating gives values of 0 20 0.25 and 0 14 ml/10 cm/hr in the 3 cases investigated It is also possible to express the leakage per sq cm of plane surface, assuming a radius of 2 cm (69) Thus the figures 0 036 0 046 and 0 012 ml/sq cm/day are obtained. No proteolytic correction factor was determined in man the maximal value for the leakage was instead estimated by *adding the non precipitable fraction* of radioactivity This fraction was assumed to consist of I amino acids derived from [31]I albumin degradation intra intestinally The fast circulation of the amino-acid pool was not taken into consideration here, nor were the desamination and deiodination that occur in the liver

In *II* are reported values for the leakage in the different parts of the intestine with corrections for proteolytic activity There are wide variations in the proportions of non precipitable ([31]I amino acids) and $AgNO_3$ precipitable ([131]I iodide) activity This is probably depending on differences in absorption of [131]*I-amino acids from the* [131]*I albumin* degradation, their deiodination and des*amination in the liver and the subsequent* excretion of some of the I iodide into the intestine By determining the average proteolysis and by using the correction factor thus derived, several difficulties in estimating the true leakage were avoided.

Thus, it has been shown that the leakage of albumin per unit length or surface area is greatest in the duodenum *next in* the jejunum, and smallest in the ileum. Significant differences are demonstrated only in the correlation of the leakage to intestinal length.

There is good correlation between the leakage into and the blood-flow to the *different parts* of the gastro-intestinal tract (II)

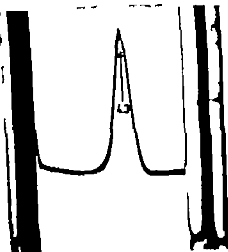
The uncorrected values for the jejunal leakage as ml plasma/sq cm/day in man, although few and in dogs (mean 0 038) are roughly of the same magnitude.

When the obtained values of the leakage are correlated to the daily catabolism of albumin, it is concluded that as an average the small intestine is responsible for about two-thirds and the stomach for one tenth or less of the catabolism.

Discussion

There are still different opinions as to the gastro-intestinal tract *being the main site* of albumin catabolism. This divergency is mostly a question of methodology Evisceration experiments (58) showed an unchanged T/2 for total-body activity and pooled plasma. *Katz* et al. (61) found a decrease of 20 % of the catabolism after enterectomy Investigations by *Franks Mosser & Anstadt* (47) and *Franks Edwards Lackey & Fitzgerald* (46) with *removal of 60—70 % of* the small intestine did not show any decrease *of the absolute* degradation rate.

Against this it may be argued that such a major surgical performance as subtotal total removal of the intestine causes extensive disturbances in the steady state. Impairment of circulation changes in intra and extra vascular distribution and *postoperative* disturbances of renal function are *factors* that may conceal or obscure the effects of organ removal per se.



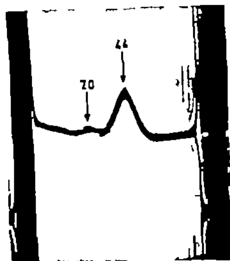
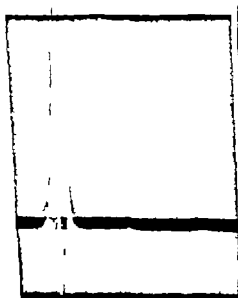


Fig. 4 Ultracentrifugation of ^{51}Cr-albumin (right) and the native albumin (left) from which it was prepared. An extra peak of 7S is observed in the labelled, but not in the unlabelled preparation.



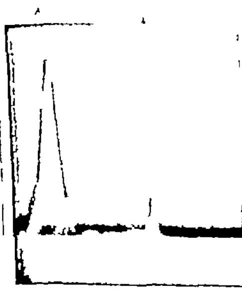


Fig. 4 b. Tiselius electrophoresis of ^{51}Cr-albumin (right) also show heterogeneity with peak at the site γ-globulin (7S) not present in the native protein (left).

CHAPTER III

^{51}Cr labelled albumin

In 1961 Waldman (170) introduced ^{51}Cr albumin as a tool for diagnosing gastro-intestinal protein loss. The reason for this was that after entering the gastro-intestinal tract ^{51}Cr is reabsorbed only to a very small extent the main part (93—98 %) leaving with the faeces (167 170 172). In normals a very small amount, about 0 1—0.5 % of the label given intravenously enters the alimentary tract in the subsequent 4 days according to *Waldman* (170 172) who states that this fact indicates that only a very small part of the normal catabolism is gastro-intestinal (1.5—4 %) In order to test the validity of this statement the following investigation was performed

Material and methods

Four healthy dogs and one man without any apparent gastro-intestinal disease served as subjects of the investigation

The ^{51}Cr albumin (Philips) used had been prepared by labelling human albumin (Behringwerke) with ^{51}CrCl$_3$ by *Waldman's* method. Free ^{51}Cr radioactivity had been removed by electrodialysis. The product contained ~25 μC per mg albumin

Ultracentrifugation and Tiselius electrophoresis of the ^{51}Cr albumin as well as of the native albumin used for labelling were performed. Dialysis and precipitation were made in order to determine the percentage of protein-bound radioactivity

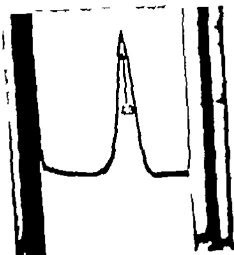
In 2 dogs intestinal perfusions and in 1 gastric perfusions were made in order to measure the leakage, using the technique described in II All were perfused for 2 consecutive hours. To investigate the leakage of simultaneously administered ^{51}Cr and ^{131}I-albumin under different experimental conditions, 400 ml of blood were withdrawn after the first hour of perfusion in the former 2 dogs. The gastric perfusion was made with physiological saline and phosphate buffer for 1 hour each.

A fourth dog was studied metabolically as was the man, in order to compare the elimination of ^{51}Cr albumin and homologous ^{131}I-albumin Urine and faeces were collected separately Two of the dogs were sacrificed 5 hours and 7 days after the dose-administration to study the distribution of ^{51}Cr radioactivity in the different organs.

Results

^{51}Cr albumin Dialysis of the label showed 1 8 % of the activity to be dialyzable. However only 46 % of the labelled material was precipitable with TCA.

Ultracentrifugation revealed that the label was heterogeneous with a peak with S_{20} = 7 except the normal one (S_{20} ≈ 4 4) This extra peak was not present in the native albumin (fig. 4 a) The Tiselius electrophoresis revealed similarly a peak at the site of γ-globulin which was not found in the native preparation (fig 4 b) About 29 % of the preparation was located in this position



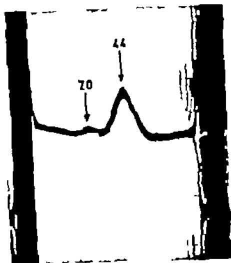


Fig. 4 a. Ultracentrifugation of ^{51}Cr-albumin (right) and the native albumin (left) from which it was prepared. An extra peak of 7S is observed in the labelled, but not in the unlabelled preparation.

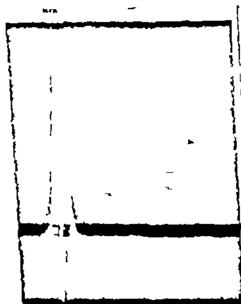




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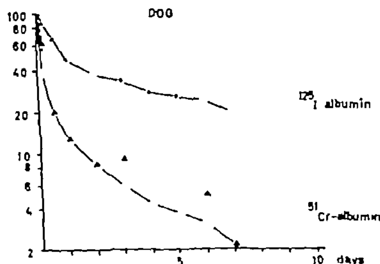


Fig. 5 b Similar study as in fig. 5 performed in dog. Symbols as in fig. 5 a.

In the dog, 2.29 % of the dose were recovered in the faeces during 7 days, in the man 1.37 % in a 5-day period.

The simultaneously determined leakages of ^{125}I and ^{51}Cr-albumin expressed as ml of plasma per 10 cm of intestine and hour and the gastric leakage per hour are tabulated in Table III. Values for ^{125}I-albumin leakage are given corrected for proteolysis as well as uncorrected.

Dog	Jejunal leakage		Ileal leakage	
	^{125}I-albumin	Cr-albumin	^{125}I-albumin	^{51}Cr-albumin
Cr 1	0.149/0.095 0.067/0.043	0.092/0.079 0.037/0.033	0.009/0.006 0.007/0.004	0.09/0.006 0.017/0.004
Cr 2	0.183/0.118 0.072/0.016	0.127/0.056 0.036/0.036	0.048/0.031 0.023/0.015	0.067/0.038 0.015/0.010

Dog	Gastric leakage	
	^{125}I-albumin	^{51}Cr-albumin
Cr 4	0.81 0.98	0 0

TABLE III. *Leakage of homologous ^{125}I-albumin and heterologous ^{51}Cr-albumin into dogs' intestine and stomach. Values expressed as ml of plasma/10 cm intestine/hour. Figures to the left of the line denote the leakage of iodinated albumin corrected for proteolysis and those to the right the uncorrected leakage. The ^{51}Cr figures denote similarly total activity and TCA precipitable activity. Note that no chromium activity leaks into the stomach.*

The metabolic studies in man and in the dog are shown in figs. 5 a and b. The ^{51}Cr albumin was eliminated from plasma very rapidly especially during the initial period. After 2 hours, only 52.4 % and 39.5 % of the dose given remained intravascularly in the man and the dog respectively as against 92.4 % and 86.5 % of the ^{125}I albumin. During the time required for intravascular mixing (9—12 minutes) 22—38 % of the ^{51}Cr albumin had already left the plasma space. This made determinations of the plasma volume with ^{51}Cr albumin impossible. *In figs 5 a and b* are also shown disappearance curves with the 100 % value calculated from

$$\frac{\text{injected dose of } ^{51}\text{Cr-albumin}}{\text{true plasma volume}}$$

From the second day the two curves have the same slope but run on different levels. Compared with ^{125}I albumin the disappearance of ^{51}Cr albumin is extremely fast.

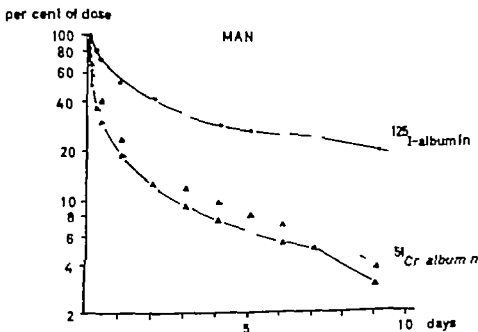


Fig. 5 a. Comparative metabolic study of homologous ^{125}I albumin and ^{51}Cr-albumin in man.
●——● plasma-activity of ^{125}I-albumin as a percentage of initial plasma-activity
▲ ▲ plasma-activity of ^{51}Cr-albumin as percentage of initial plasma-activity (10-minute sample)
▲——▲ plasma-activity of ^{51}Cr-albumin as a percentage of "ideal" initial ^{51}Cr-activity (= dose ^{51}Cr-albumin/true plasma volume)

ed amount is regained. A further reason for this may be that a 4-day collection of faeces does not catch the whole delivery of ^{51}Cr. In the dog, maximum radioactivity was recovered during days 5—7.

As the determinations of the amount of ^{51}Cr and ^{131}I-albumin in the perfusion fluid were made on the same samples, it is quite evident from the figures in Table III that the ^{51}Cr preparation does not behave like the ^{131}I-preparation, neither in the jejunum nor in the ileum. Another fact which shows the different natures of ^{131}I-albumin and ^{51}Cr-albumin is that the latter will not pass into the stomach. It has been convincingly shown by several authors that albumin leaks into the stomach (20, 61, 73, 80, and papers I—II).

In conclusion, it can be stated that ^{51}Cr labelled albumin is a heavily denaturated protein which does not behave like iodinated or native albumin. Thus, no statements as to the normal degradation of albumin can be made on the basis of investigations with ^{51}Cr-albumin.

For ^{51}Cr-albumin the first figure is derived from total activity and the second from the TCA precipitable fraction. It will be seen that, with the exception of the ileum in dog 1 the leakage during the second perfusion is evidently less than during the first one. The leaked-out protein-bound ^{51}Cr radioactivity expressed as ml plasma/ 10 cm intestine/hour is of the same order of magnitude as that of ^{131}I-albumin uncorrected for proteolysis. When corrected the figures exceed those for total ^{51}Cr activity in the jejunal but not in the ileal samples The gastric leakage of ^{131}I albumin is equivalent to 0 8—1 ml of plasma/ hour No ^{51}Cr activity at all was found in the gastric perfusates.

It was evident that a certain fraction of the ^{51}Cr-albumin was trapped in the liver (5 % of the dose or 17 % of retained dose) and spleen (1 % of the dose) still after 7 days Also in the lymphatic glands the activity/g tissue was very high equivalent to 1 1 ml of plasma. Even after 5 hours there was considerable activity in the liver (3 % of the dose) It was also obvius from both dogs that a certain fraction of the ^{51}Cr-activity enters the contents of the gut

Discussion

The main reason for using ^{51}Cr-albumin in tracing intestinal protein losers is the inability of the intestine to reabsorb the ^{51}Cr ion, once it has entered the intestinal lumen Of intravenously given ^{51}CrCl 3 % appear in the stools in 7 days (167) and of ^{51}Cr albumin a maximum of 0 7 % in 4 days (170)

In spite of electro-dialysis of the label, there is an extensive divergence between the fractions of dialysable and TCA precipitable Cr radioactivity Thus heterogeneity is further stressed by the ultra centrifugation and Tiselius electrophoresis results. The presence of a 7S-peak suggests a molecular conglomeration. *Gray & Frank* (70) showed that ^{51}CrCl given intravenously had a specific affinity for plasma proteins. In this investigation however the very rapid disappearance of the label from the circulation indicates that a considerable portion of the ^{51}Cr albumin is immediately taken care of by the reticuloendothelial system. This is evidenced by the rather high uptake in the liver spleen, and lymph glands. The removed fraction obviously consists of heavily denaturated protein. With this rapid initial disappearance the plasma activity curve will naturally become still steeper and lower when plotted as a percentage of the dose. As the degradation of ^{51}Cr albumin, being a denaturated protein, is much faster than that of ^{131}I-albumin an abnormal mechanism is engaged in the process of degradation. This means that only a fraction of the intravascular ^{51}Cr albumin is available for the normal catabolism.

That a fraction of the ^{51}Cr-albumin is able to pass through the intestinal mucosa is evident from the present investigation

If the expected faecal regain of activity is calculated in the two metabolic studies, assuming that two-thirds of the catabolism occurs in the gastro-intestinal tract, the obtained figures will be 4.55 % in the man and 6 25 % in the dog provided that all ^{51}Cr-activity in plasma represents undenaturated ^{51}Cr albumin. The observed values were 1.37 % and 2.29 %. This means that about one third of the expect

catabolism too. Formulas for estimating body-surfaces in the different species are available (147)

The encountered values for g of albumin catabolized per sq m and day in the different species are plotted in fig 7

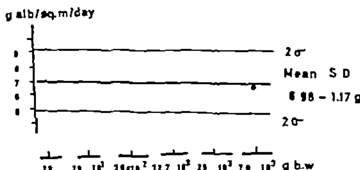


Fig 7 Albumin catabolism (g) per sq m body-surface area and day plotted against body weight. The slope of the line does not differ significantly from zero. ± 2 are marked out and mean ± S.D is given.

On an average, 6.98±1.17 (mean±S D) g/sq m/day are degraded in the species investigated. The slope of the regression line does not differ significantly from zero. Evidently the catabolism per sq m body-surface area is quite independent of the body-weight.

TABLE IV *Degradation of albumin in 5 different species (mammals) expressed in % of intravascular pool and in g/kg body-weight Values for rat and mouse are calculated from data by authors listed below Calculated figures for degradation (g) per sq.m body surface area and day are set out in the right column.*

Species	% of intravascular pool/day	g/kg b. w/day	g/sq m body-surface area/day
Man	9-10	0.17 0.19	6.8
Dog	17 18	0.30 0.31	6.1 7.6
Rabbit	21 23	0.31 0.35	5.4
Rat	50-60	1 1	6.1
Mouse	150	2.4	7.8

1) Table I this paper 2) P per III 3) P per 1 I agreeing with those of Cohen et al. (1956) Rees and Robert (1958) Matthews (1957) and McFarlane (1963) 4) Campbell et al. (1956) and Matthews (1957) 5) Dixon & Weigle (1957) and Friedberg (1963)

The nature of the catabolic process

As mentioned before, it has been discussed whether the catabolism is a zero or first order process The study by *Friedberg* (54) in albumin loaded mice lends strong support to the first order process. So do the works by *Matthews* (104 106) and *Franks Mosser & Anstadt* (47) This problem is of primary interest as it also includes the question of the interrelation between breakdown and synthesis of albumin.

A relationship between the turnover time of serum proteins and the third root of the body-weights in different species has been postulated by *Niklas & Maurer* (119)

Own investigations

Another approach to this problem will be introduced.

There exists a linear relationship between blood volume and body weight (2, 145) The plasma volumes in ml/kg of body weight are fairly equal throughout the different species (mouse — rat — rabbit — dog — man) roughly varying in the range 38—53 ml/kg So the intravascular amounts of albumin will be 1.5—2 0 g/kg body weight. This conformity is as striking as are the differences in albumin catabolism between the species. Table IV demonstrates this, and in terms of *quantity* the catabolism varies within the range 0 17—2 4 g/kg body weight with an *inverse relationship* between the quantity catabolized and the body weight. A double logarithmic plot of mg albumin catabolized per day and average body weights in g of the species shows a linear regression (fig 6)

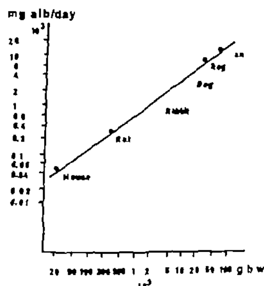


Fig 6. Double logarithmi plot of mg albumin catabolized per day against body weight in different mammals. The equation of the line i given in the text.

The correlation is highly significant with $r = 0\ 996$ The equation of the line is

$Y = 5\ 83 \times X^{0.884}$

A similar regression is valid for average heat production and body-weight (25 Benedict cited by ref 130) The power of the body weight is 0 73 (25)

As heat production follows the surface rule" (96) this seems to apply to albumin

The intestinal capability to deliver own (endogenous) proteins was first demonstrated by *Dreisbach & Nasset* (41). These proteins provide a wide supply of different amino acids with the object of preventing too great fluctuations in the mixture of amino acids available for absorption (115, 117). According to *Nasset* about 100—150 g of endogenous protein are thus delivered daily in man. The normal albumin leakage into the intestine is with all probability an integrating part of this mechanism. The leaked-out albumin obviously constitutes about 10 % of that amount of endogenous protein. It seems then quite logical that, referred to body weight, the leakage is highest in small, metabolically more active, animals. *Nasset* also states that alimentation stimulates the transfer of endogenous proteins, while starvation fairly quickly impairs this homeostatic mechanism.

Another aspect of the expediency of the gastro-intestinal catabolism of serum albumin and its service in supplying amino acids is shown in an investigation by *Holm* (79). Intravenous or intraportal infusions of casein-hydrolysate (Aminosol®) during 4 weeks to dogs fed a protein-free diet were attended with hypoalbuminaemia. This was not the case, however, when the hydrolysate was given orally or enterally. These observations may indicate that the intestinal mucosa also plays a significant role in "adapting" the amino acids to the synthesis of proteins — at least albumin — in the liver.

The purpose of this metabolic character obeying the "surface law" is not quite clear. Albumin is responsible for the main part of the oncotic pressure of plasma. It also serves as carrier of a lot of substances and metabolites, participating in different metabolic processes in the organism. As far as we know today however this function does not necessitate degradation of the albumin molecule. This carrier function of albumin will be discussed later in connection with the capillary transfer.

The theory proposed and partly evidenced by *Friedberg* et al. (53) and *Haurowitz* et al. (75) of albumin as a reserve protein available for intracellular protein synthesis after catabolism fits fairly well with this metabolic behaviour. This indicates that the breakdown takes place in close connection with an active proteolytic enzyme system. That no catabolism occurs in plasma, whole blood or red cells is shown by *Reeve & Roberts* (133).

In what way do the albumin and the actual proteolytic system meet? As the *first order process is the most probable*, the concentration of albumin, and not the strength of the proteolytic system, seems to determine how much albumin is broken down per unit of time. This question is obviously closely bound up with that of where the albumin is catabolized.

The fairly good correspondence between the intestinal surface leakage in man and in dogs may be indicative of an equality in capillary transfer per unit *intestinal surface area*.

The true mucosal area is impossible to estimate. No accurate information on the surface enlargement because of plications and villi in the different species is available. The plane intestinal surface area, however may be roughly calculated. As seen from Table V a certain conformity exists as to intestinal surface area per sq.m body surface.

Table V. *Calculat ns f plane intestinal surface ea n th d fferent species eferred t body-weights and body-surface areas*

Species	Length of small intestine in cm	Diameter of small intestine in cm	Intestinal surface area (sq.cm) per kg body-weight	Intestinal surface area (sq.cm) per sq.m body-surface
Man	~ 310	3 4	~ 45	~ 2000
Dog	300 450	1 2	~ 90	1900
Rabbit	200 300	0 4 0.6	100 190	1700 3000
Rat	90 110	~ 0.25	200 300	~ 3000
Mouse	25	~ 0.16	625	~ 2100

1) Hirs h et al (1956) 2) Grey (194) 3) Own measurements = 20 4) Idem = 15 5) Fisher & Par ns (1950) 6) Own measurements n = 10 7) J ffé (1951)

Thus, it does not seem incoherent to assume a fairly uniform albumin leakage per unit plane intestinal surface area throughout the species examined.

Distribution and capillary transfer of albumin

Distribution

Previous investigations

The extravascular distribution of albumin is well-established fact (1 10 18, 28, 32, 35 60 82, 152) As to the ratio extravascular to intravascular albumin, different figures are obtained depending upon the methods used by the different authors. The figures for this ratio vary between 1 and 2. Generally four methods (described in III) may be used for this purpose. *Berken* et al. (11) showed in man that the methods gave fairly diverging values.

An alternative to these methods is the determination of the specific albumin content in the organs, a way that has been used by some authors (38, 103, 123, 138)

The graphical mathematical treatment and the interpretation of curves and figures obtained in work with tracers in biological systems have been extensively studied and analyzed by several authors.

The kinetics of serum albumin are best described in an open mamillary or catenary system. Problems concerning this are treated by *Berman & Schoenfeld* (17) *Matthews* (103) and *Lewellen Berman & Rall* (99) Further adaptations with some modifications of the compartment system have been made by *Reeve & Roberts* (133 134) *Takeda & Reeve* (137) and *Reeve & Bailey* (131) Recently *Nosslin* (121) has designed a new mathematical approach to the kinetic analysis, not yet published. *Bergner* (16) has thoroughly reviewed the fundamentals and discussed the errors of assumptions generally made in works on tracer kinetics.

Matthews (103) found two extravascular compartments in man as well as in some rabbits, while in the latter species *Reeve & Roberts* (133) were not able to derive more than one.

Matthews (103) showed that the small and more rapidly exchanging extravascular pool consists of the gastro-intestinal tract and the parenchymatous organs, while the larger pool with a slower exchange comprises muscle, skin, bone, and f t.

Own investigations

The normal elimination of ^{131}I albumin from plasma with slopes and intercepts of the exponentials in dogs and rabbits is shown in III and VI respectively A comparison between the results obtained by others and the author's results for man (mean values) is made in Table VI.

The corresponding figures for rabbits are given in Table VII In 3 out of 6 dogs

CHAPTER V

Synthesis

That albumin is synthesized in the liver is well documented (89 115) There does not exist any objective reliable method for measuring the synthesis. Recently *Reeve Pearson & Mart* (132) and *Mc Farlane* (114) have used arginine labelled with ^{1} C at the guanidine group But this method can only be used with long intervals and does not reflect fast changes in the synthesis rate.

The only way of estimating the synthesis is at present by indirect evaluation. Steady state is necessary for this. At constant albumin concentration and plasma volume, the synthesis equals degradation.

Evaluation of the synthesis under pathological non-steady state conditions will be discussed later

the main part of the total extravascular albumin pool of the body

Discussion

Comparison between the figures that form the basis for compartment analysis, the slopes (λs) and the intercepts (Cs) obtained by different authors in man and in rabbits reveal a good conformity as to the last exponential (λ C_1). But apparently the agreement is not so good in the early stage, where one or more exponentials with high λs are predominating. Evidently a closer analysis of the early distribution period is necessary to discern most exponentials.

As seen from the author' dog experiments (III) the analysis of the curves offered difficulties in some of the animals. Only in man, is there no uncertainty as to the presence of three exponentials. However the numerical values of the two first exponentials show considerable variations.

Physiologically there exists no explanation of the finding that small species seem to have fewer extravascular compartments than do large species.

A divergency between the curves for plasma activity and total-body activity was first shown by *Lewallen, Berman & Rall* (99) in their tw cases. This finding is further verified in the present control cases. This divergency indicates the presence of one or more albumin pools with very slow exchange with the intravascular pool. One argument against this interpretation of the results will always arise, namely that of incomplete urinary collections. This objection must also infer that the urinary collections should be most incomplete in cases with gastric cancer (IV) especially those with ascites. Such a coincidence seems highly improbable. The definite answer will be achieved by the use of a whole-body counter with high efficiency. We are now making such investigations. The disclosure of these pools in man is probably explained by the fact that this species has a lower catabolism and also a lower capillary transfer per unit body-weight (see next chapter). So the pools with a very slow rat f exchange will have time to ppear before the radioactivities have reached too low levels and while the measurements are still signifi cant. Bergner (16) has suggested that this is an expression of time lumping

The different methods for calculating the extravascular albumin pools are discussed in III.

The equilibrium time method (28) has been used in the clinical investigations on the assumption of one extravascula pool Although the regarding of all extravascular pools as one homogeneous pool is a simplification, this method is advanta geous in clinical practice. In man this method does not reveal the slowest ex changing pools, which appear later in the course of the investigation.

By compartment analysis it possible to obtain pool masses and rates of ex change under steady-state conditions (III). By this method it is possible to fit the emparic curves to the experimental values. However *Bergner* states "A compartment can be chosen at will and lacks therefore any physical significance of its own. It is therefore obvious that a model using such compartments as basic entities *is not likely to have much physical signif icance* As is partly discussed in III and will be discussed later on, the different

TABLE VI. *Slopes (λ_1—λ_3) and intercepts (C —C_3) obtained by graphical analysis of plasma-activity curves in man according to different authors $\Sigma\lambda$ C_n (= the sum of capillary transfer and catabolic rates) are listed in the right column.*

Autho s	λ	λ	λ	C_1	C_2	C_3	Σλ C_n
Matthews 1957	0 0302	0.215	2 77	0.38	0.146	0.474	1.364
Cohen et L 1961							1 60
Beeken et al 1962	0 0473	0 579	3.34	0 356	0.346	0.298	1.212
Takeda & Reeve 1963	0 0357	0.537	2 447	0.383	0.216	0.401	1 119
Wetterfors 1965	0 0391	0.562	2.24	0.374	0.350	0.276	0.859

TABLE VII *The same units as those in t ble VI obtained rabbits by d fferent authors.*

Author	λ	λ	λ	C	C	C_3	Σλ C_n
Matthews 1957	0.080	1 00	14 4	0.33	0.53	0 14	2.57
Reeve & Roberts 1959	0.0865	1 497 2 165	—	0.346	0.654	—	1.259
Cohen at L 1956	0 072 0 090	—	—	0.41 0.46	0.54- 0.59	—	—
Wetterfors et al 1965	0 088	1 77	—	0.37	0.63	—	1 12

three exponentials indicating two extra vascular compartments could be derived. The second exponential must, however be judged very critically as the differences between the extrapolation line from the linear part of the exponential curve and the rest of the curve were very small In the rabbit it was possible to trace only one extravascular compartment.

In man, the largest species investigated, three exponentials (two extravascular compartments) were always distinguishable. In IV is discussed a phenomenon appearing in the [131]I albumin studies in man. In the control cases investigated at that time there is a divergency between the plasma activity and retained dose curves. This divergency is expressed as the quotient between the slopes of the re spective curves. (K_{Pr} /K_{rt} or λ /λ retained dose) with a mean (± SD) of 1 49 (± 0 19) This finding is indicative of one or several slowly exchanging extra vascular albumin pools.

The four methods for estimating the size of the extravascular albumin pool are compared in III It is shown that there exists a divergency between the methods as well as between the individuals.

The extravascular albumin content per g wet tissue (specific albumin content) determined by the double isotope tech nique, in different organs of the dog is presented in III. The gastro-intestinal tract has the highest extravascular albu min content per g tissue, while the paren chymatous organs, with the exception of the lungs, contain small amounts. The extravascular albumin in the muscles (3.5 —4 0 mg/g tissue) obviously constitutes

of the total capillary transfer

Is this mathematical exactness matched by a corresponding exactness in the performances of the investigations? This may be doubted when one compares the different data obtained as well as the different numbers of compartments derived, and particularly when taking the very fast transfer processes into consideration.

Own investigations

In papers III and VI are given the values for $\Sigma\lambda$ C (sum of capillary transfer and catabolic rates) in dogs and rabbits. The experimentally determined initial disappearance rates ($\lambda_{1\rightarrow 2}$) for each species are also given there. For man and rabbits the corresponding $\Sigma\lambda$ C_n are tabulated in Tables VI and VII with values achieved by others. In man they range between 0.838 and 1.364 and in rabbits between 1.12 and 2.37.

In 4 individuals the initial disappearance rate was determined by the methods described in III and VI. Fig. 8 shows the disappearance curve for one of the volunteers examined. The $\lambda_{1\rightarrow}$ varied between 1.5 and 1.9 with a mean of 1.77. $\lambda_{3\rightarrow}$ was also determined, but will not be accounted for here as no conclusions are drawn from these figures at the present time.

The differences between the $\Sigma\lambda$ C_n and the experimental figures in the three species investigated are obvious, as can be seen from Table VIII.

However, as this difference could possibly be due to a certain protein denaturation, with a rapid uptake of the denaturated molecules by the reticuloendothelial system, a similar experimental procedure was performed in 3 rabbits (the species where the differences were greatest) with a homologous ^{131}I albumin preparation screened in another rabbit for 3 days. With this purified preparation, administered as heparinized plasma (2—3 ml) the obtained values of the initial disappearance were of the same magnitude and within about the same range as those of the unscreened label $\lambda_{1\rightarrow}$ was 2.60, 3.26, and 4.10 (mean 3.32).

TABLE VIII. *Graphically determined values for total capillary transfer ($\Sigma\lambda$ C_n) in the different species according to different authors. Comparison is made with the experimentally determined values (initial disappearance rate). Figures achieved by screened preparation of homologous ^{131}I-albumin in rabbits are also listed. In rats heterologous preparation was used.*

Authors	Man	Dog	Rabbit	Rat
Matthews 1957	1.36		2.45	2.07
Reeve & Roberts 1956			1.25	
Becken et al. 1962	1.21			
Takeda & Reeve 1963	1.12			
Takeda 1964		1.56		
Wetterfors 1965	0.84	1.09	1.12	
Experimental values	1.77	2.01	3.83	5.02
Screened preparation (Wetterfors 1965)			2.60 3.26 4.10	

flow rates obtained by this analysis may not bear any significance, since the total capillary transfer generally seems to be underestimated. However, the problem is not only how to treat the results obtained but also how exact the procedures are through which the actual values are achieved.

Determinations of albumin distribution in different organs by the *double-isotope* technique give values of fairly good correspondence. However, the variations in organ weights may have great influence in the estimations of the total extravascular pool. Another difficulty is to calculate the extravascular as well as the intravascular albumin content in heterogeneous tissues such as the subcutis, fat, and bone, the total weights of which are impossible to estimate with any accuracy.

Capillary transfer

Previous investigations

The total capillary transfer rate for albumin in man is generally stated to be 1 % of the intravascular pool per 6 minutes (44, 166) or 8 % per hour (120). These rates are all-over values for the whole organism. As early as 1909 *Starling* (148) *was aware that capillaries had different* qualities as to permeability depending on their organic location. *Netsky & Lester* (118) proved that the capillary permeability for proteins is greater in the areas drained by the thoracic duct. I albumin appears in the thoracic duct lymph within 10 minutes of intravenous administration of the label. After 7—13 hours the specific activities of the plasma and lymph are equilibrated (173).

The increase of the concentration of labelled protein is fastest in hepatic lymph, next in intestinal lymph, and slowest in muscle (109). *Abdou & Tarver* (1) *found* a high residual activity in the liver, spleen, and kidneys after "wash out" within 4—10 minutes of administration of serine-β-C-labelled plasma proteins intravenously. In other organs there was less activity. They conclude " ... *that it is either* mechanically impossible to remove all the *plasma from* these tissues by the in vivo perfusion technique or that the plasma protein enters the extracellular or intracellular space at an extraordinarily rapid rate."

Other evidence of the fast disappearance of albumin is the differences in plasma volume determinations made with ^{131}I albumin and ^{131}I fibrinogen or when using values obtained from red-cell-volume measurements. The "albumin space" measured is consistently 10—15 % larger than that for fibrinogen, or that derived *from the red-cell space* and body haematocrit (7, 8). The investigations by *Strenn, Stegall, Collings & Miles* (155) concerning the disappearance of ^{51}Cr labelled red-cells and ^{131}I-albumin *from the ileum* further stress the fast disappearance of albumin.

One of the physiological factors that forms the basis for compartment analysis is the differences in capillary transfer in different organs or organ systems. This transfer is based on the sizes and number of pores present in all capillaries (72, 109). However, some evidence exists which makes an active process with the participation of enzymes and structural changes probable (26).

Matthews mathematical expression for the capillary transfer and catabolic rates (formula in III) is an empiric definition

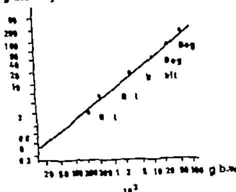


Fig. 9 Double logarithmic plot of total capillary transfer of albumin (g) against body weight in different mammals. The equation of the line is given in the text.

By calculation in relation to body-surface area it is shown that about 60—150 g of albumin pass the capillary walls per sq.m body-surface area per day in all species (Table IX). The average value is 96 ± 18 g (mean ± SD) assuming an intravascular pool of 1,8 g per kg body weight. The capillary transfer per sq m body surface is independent of body weight (fig. 10).

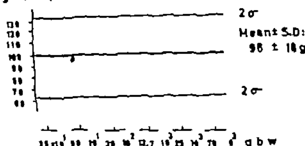


Fig. 10 Capillary transfer of albumin (g) per sq m body-surface area and day plotted against body-weight. The slope of this line does not differ significantly from zero. ± 2 are marked out and mean ± S D is given.

Discussion

It is generally believed that the return of extravascular proteins to the blood occurs entirely in the lymphatics (see ref 176). *A esk g Arth von Grotte & Wallenius* (4) have shown that in the heart the return of extravascular protein is to at least 90 % taken care of by the lymph. The lymph-flow through the thoracic duct erage 1—2 ml/kg/hr in all species and constitutes 70—90 % of all lymph returning to the circulation (see ref 176). This means that the lymph-volume delivered to the blood per day approximately equals one plasma-volume. If this is about 50

In 5 rats the $\lambda_{t=0-2}$ was also determined by methods described in III and VI

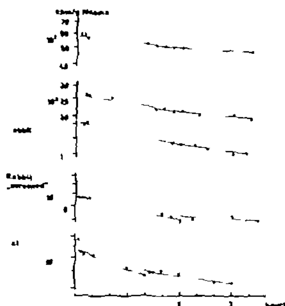


Fig 8 Initial disappearance of homologous iodinated albumin in man, a dog and rabbits. In the rat iodinated human albumin was used $\lambda_{t=0-2}$ man 1.83 dog 2.24 rabbit 3.06 rabbit, screened 3.26 $\lambda_{t=0-}$ rat 5.54

Small siliconized polyethylene catheters were introduced by a special technique (24) into the external jugular vein and the aorta one week prior to the investigation in 4 of the animals, and just before the investigation in 1 animal. Human ^{131}I-albumin was administered through the venous catheter and sampling of whole blood (0.05 ml) done from the aorta catheter for 3 hours with intervals as described earlier

$\lambda_{t=0-2}$ ranged between 4.5 and 7.0 with a mean of 5.82.

It is evident that smaller species have greater initial disappearance rates. As the intravascular albumin content per kg of body weight is fairly constant throughout the species, the differences in registered disappearance rates mean that the total intra- to extravascular transfer per unit body weight is greater in the smaller metabolically more active, animals. By multiplying the experimentally determined disappearance rate by the intravascular albumin mass, the total capillary albumin transfer will be obtained (Table IX)

TABLE IX. *Mean initial disappearance rates for 4 investigated species S.E.M is put in parentheses Capillary transfer of albumin in g is listed as total amount per day and correlated to body-weight and body surface area*

Species	Intravascular albumin g/kg b.w	Initial disappearance rate Mean (±S.E.M)	Capillary transfer of albumin		
			g/day	g/kg/day	g/sq.m/day
Man	1.8	1.77 (± 0.09)	225	3.2	124
Dog	1.8	2.01 (± 0.35)	46-90	3.6	75-94
Rabbit	1.8	3.83 (± 0.42)	21	6.9	112
Rat	1.8	5.82 (± 0.49)	2.6-5.4	10.8	73-98

A double logarithmic plot (fig 9) of these values against body weights shows a highly significant correlation (r = 0.973) The equation of the line is

$$Y = 0.028 \times X^{0.89}$$

participation of the endothelial cells as well (12, 2b) Certain electron-microscopic and microphotometric investigations speak in favour of the latter opinion (12, 23)

Whichever alternative or alternatives are valid, they indicate a greater exchange through the capillary wall than is hitherto supposed to exist, and, furthermore, when correlated to the total intravascular pool this exchange is greater in smaller metabolically more active, animals (mammals)

The significant log-log correlation between capillary albumin transfer and body weight, and the quantitatively constant transfer of albumin per sq.m body-surface area, independent of species, are facts indicative of a metabolic purpose. Such a purpose is its function as carrier of different metabolic substances and metabolites. It can be easily shown that the catabolism constitutes a certain fraction of the total capillary transfer viz. 5—10 % in all species examined.

The correlation of capillary transfer to body mass raised to the power of 0.629 in the different species is in analogy with that of oxygen consumption (heat production) to body mass (power 0.66—0.75) and cardiac output varies accordingly As the blood pressure (systolic) is known to be fairly uniform among the mammals, the blood-flow varies. It has been shown (vide ref. 145) that the blood flow is proportional to the surface area of the peripheral vessels (capillaries) which means an increase in number and width of the capillaries per unit of tissue weight. Hence, the blood-flow as well as the capillary surface area are adapted to satisfy enhanced basic metabolic demands.

This fast capillary transfer is not revealed in any model hitherto used for compartment analyses. This is a very good demonstration of what *Bergner* calls "time-lumping" Evidently a closer analysis of the initial stage of the distribution period is necessary to obtain a true picture of the capillary transfer process, and a more exact compartment model. Investigations concerning these problems are in progress.

ml/kg The protein content varies depending on the area drained but is always, as a total lower than for plasma. In conclusion this means that generally the albumin mass returning to the circulation by the lymphatics amounts to 75—90 % of the intravascular pool. Since protein leaving the circulation for the extravascular pool must be compensated for by an equivalent return to maintain steady state, this would expressed as initial disappearance rate, give a $\lambda_{\rightarrow 0}$ of about 0.75-0.90 for all species. As is seen from the results, this value is not valid for any species.

This problem concerning the lymphatic protein return is dealt with from a different viewpoint by *Rieger Liljedahl Plantin & Birke* (136) viz. *the importance of* the lymphatics in the restitution of the intravascular protein pool after bleeding

The discrepancy between the values calculated from lymph return and those experimentally determined may be explained alternatively

A Denaturation of the labelled protein *with increased disappearance because of* uptake by the reticuloendothelial system.

B The measurements of lymphatic flow are incomplete

C. There are lymphatico-venous connections other than the thoracic duct, the right lymph duct, the cervical ducts, and those from the extremities.

D There are routes for the return of extravascular protein other than the lymphatics.

Denaturation as a cause of the obtained values for initial disappearance is excluded by the tests with a screened preparation in rabbits and also by the very fact that the figures are successively increasing in the smaller animals. Human labelled albumin gave "low" values in man, but "high" values in rats, *which were not immunized* and in which no reaction of immunity was to be expected during the short period of investigation (40) This was one reason why human [131]I-albumin was used in the rats.

As to the second alternative most papers on this subject agree as to a thoracic *duct lymph flow of 1—2 ml/kg/hr* However this would mean that in man a little more than half and in the rabbit about one-third of all the protein return would be accounted for by the lymphatic route

Alternative C is not to be disregarded as there are many reports indicative of the *presence of lymphatico-venous connec*tions other than those mentioned above (22 48 90 98 143) Reviewing these reports, it seems as if the "extra" lymphatico-venous connections were more numerous in the smaller than in the larger animals. This fact may well agree with the higher $\lambda_{\rightarrow 0\ 2}$ in the former

In III is discussed the existence of a paracapillary space with a fast exchange with the intravascular pool and which is included *in* the plasma volume determined. Further support for such a space are the comparative studies by *Baker & Wycoff* (8) and *Swann* et al. (155)

Such a rapid capillary transfer necessitates an equally rapid return of the protein a fact that actualizes the fourth alternative, the active participation of the endothelial *cells in* a transcapillary inflow of protein as well

A question still under debate is whether the transcapillary passage *of macromolecules* occurs through "intercellular gaps and pores" (126 135) or by an active

CHAPTER VII

Catabolism of albumin

Gastric cancer

In 1936 *Kimbel Henkel & Börner* (94) using [131]I-albumin, demonstrated increased fractions of radioactivity (protein- and non-protein-bound) in the gastric juice from patients with various gastric diseases. They did not draw any definite conclusions from their observations, but only deduced that the method might be a useful diagnostic tool. In 1959 *Burke Liljedahl Plantin & Wetterfor* (20) found increased amounts of [131]I-albumin in gastric juice from subjects with gastric cancer and also showed an increased catabolic rate. In the same year *Schwartz & Jarnum* (141) reported elevated figures for the [131]I PVP content in the stools of patients with this disease.

In paper IV are reported the results of a more thorough investigation into the hypoalbuminaemia and its possible genesis in cancer of the stomach

As to the methods for quantitative analysis of the gastric juice they involve the same difficulties as those reviewed in I and II. The precipitability of the radioactivity varied within wide ranges, but it is evident that the highest protein-bound fractions were in the achlorhydric cases. In comparison with achlorhydric controls the values were also elevated, indicating a true increase not only one depending on the absence of peptic activity

In case 14 with complete retention of the gastric secretions, the pathological leakage of albumin into the diseased stomach was evidently much greater than normally and may very well have accounted for the whole increase of the catabolism.

On the other hand, in the normo-hypochylic cases, the different fractions of radioactivity did not deviate to any extent from those of the controls and, thus, it cannot be stated that any increased leakage of albumin occurred in these patients. It is seen from Tables IV and V b (paper IV) that the catabolism is elevated in only 2 of the 5 normochylic subjects (cases 3 and 18)

One aspect not accounted for in IV is the relations between the leakage and the histological type of the tumour However attempts at correlating the leakage to the type of tumour do not give any positive information. There are scirrhous as well as adenomatous tumours with or without achlorhydria. Hitherto, the only quality of the tumourous stomach that seems to be of any importance to the leakage is its acid producing capacity

Further reports on the increased leakage of albumin have since been published by *Haskell* et al. (76) and *Turner et al.* (164)

It is obvious from the results that the catabolic rate never reaches extreme

Part III

ALBUMIN METABOLISM UNDER SOME PATHOLOGICAL CONDITIONS

Introduction

Earlier in metabolic studies of idiopathic hypoalbuminaemia the fate of infused albumin was studied (3) The results suggested a rapid breakdown of the infused albumin It was generally concluded from these studies that part of the albumin was "burned and the rest "converted" to body proteins. Using proteins and amino acids labelled with ^{35}S an increased breakdown as well as an increased production of plasma proteins were demonstrated in a case of idiopathic hypoproteinaemia (95) Further investigations of that kind, but with ^{131}I albumin (142) verified the high catabolism of serum albumin in idiopathic hypoproteinaemia.

Nothing was known about the mechanism of this increased breakdown until *Gordon* (65) in 1958, introduced ^{131}I labelled PVP as an indicator of gastrointestinal loss of macromolecules. *Citrin Sterling & Halsted* (31) *Gordon* (66) and *Schwartz & Jarnum* (141) were the first to bring forth evidence of the pathophysiological and clinical connection between idiopathic hypoproteinaemia and certain gastropathies evidently associated with great gastric loss of protein. *Holman Nickel & Sleisenger* (80) and *Gordon Bartter & Waldman* (67) in 1959 contributed investigations into cases with enteric loss of protein. A thorough review of the protein losing gastroenteropathies is given in a monograph by *Jarnum* (85)

In 1959 (20) the interest was focused on certain more common gastro-intestinal disorders associated with hypoalbuminaemia, such as cancer of the stomach, ulcerative colitis, and regional enteritis, all being diseases of surgical as well as medical concern. Other pathological conditions of interest as to the behaviour of serum albumin are intestinal obstruction and the acute radiation-syndrome with its gastro-intestinal engagement.

These different diseases will be dealt with together under main headings referring to catabolism, synthesis, and distribution.

CHAPTER VII

Catabolism of albumin

Gastric cancer

In 1956 *Kimbel, Heinkel & Börner* (94) using I albumin, demonstrated increased fractions of radioactivity (protein and non-protein-bound) in the gastric juice from patients with various gastric diseases. They did not draw any definite conclusions from their observations, but only deduced that the method might be useful diagnostic tool. In 1959 *Bele, Liljedahl, Plantin & Wetterfors* (20) found increased amounts of ^{131}I-albumin in gastric juice from subjects with gastric cancer and also showed an increased catabolic rate. In the same year *Schwartz & Jarnum* (141) reported elevated figures for the ^{131}I PVP content in the stools of patients with this disease.

In paper IV are reported the results of a more thorough investigation into the hypoalbuminaemia and its possible genesis in cancer of the stomach.

As to the methods for quantitative analysis of the gastric juice they involve the same difficulties as those reviewed in I and II. The precipitability of the radioactivity varied within wide ranges, but it is evident that the highest protein-bound fractions were in the achlorhydric cases. In comparison with achlorhydric controls the values were also elevated, indicating a true increase not only one depending on the absence of peptic activity.

In case 14 with complete retention of the gastric secretions, the pathological leakage of albumin into the diseased stomach was evidently much greater than normally and may very well have accounted for the whole increase of the catabolism.

On the other hand, in the normohypochylic cases, the different fractions of radioactivity did not deviate to any extent from those of the controls and, thus, it cannot be stated that any increased leakage of albumin occurred in these patients. It is seen from Tables IV and V b (paper IV) that the catabolism is elevated in only 2 of the 5 normochylic subjects (cases 3 and 18).

One aspect not accounted for in IV is the relations between the leakage and the histological type of the tumour. However attempts at correlating the leakage to the type of tumour do not give any positive information. There are scirrhous as well as adenomatous tumours with or without achlorhydria. Hitherto, the only quality of the tumourous stomach that seems to be of any importance to the leakage is its acid-producing capacity.

Further reports on the increased leakage of albumin have since been published by *Heiskell* et al. (76) and *Turner* et al. (164).

It is obvious from the results that the catabolic rate never reaches extreme

values, not even in the inoperable cases. This fact is consistent with the general character of the hypoalbuminaemia of not appearing suddenly or becoming too excessive. It has earlier been made clear that the catabolic process is a first order process, i.e. it occurs on a percentage basis. As regards pathological leakages, this may not be a general rule, but an observation by *Engström* et al (43) indicates that we are concerned with a first order process at least in some diseases.

There is no consistent evidence of the hypothesis that albumin is "cannibalized" by the tumour cells. That albumin is degraded by the tumour and the degradation products excreted is a possibility which cannot be wholly excluded. Sufficient incorporation of albumin molecules by the cells would give increased activity in the tumour tissue, but this was not the case in the examined specimens. In vitro but not in vivo, some uptake of albumin by certain experimental tumour cells has been demonstrated (139 161) but mostly by surface adsorption (140).

The increased gastric leakage of albumin in patients with achlorhydric gastric cancer might probably be due to a direct loss from the neoplastic tissue or to loss from the entire gastric mucosa.

Ulcerative colitis and terminal ileitis

The first authors who dealt with the protein-loss aspect of ulcerative colitis were *Welch Adams & Wakefield* (174) by nitrogen balance studies, and *Steinfeld Davidson & Gordon* (150) by using labelled albumin.

In paper V is given further evidence of these losses and their correlation to the clinical picture of the disease. The fast release of the losses from the colon makes the calculations easier than in any intestinal disease located more proximally. It is shown that the degradation of albumin as well as the hypoalbuminaemia are more pronounced in cases with acute relapses. In these cases more albumin is lost per day in the stools, while the losses in the non-acute cases are fairly moderate. By exclusion of the faecal losses, it is possible to calculate the "true" degradation, i.e. that fraction which depends on the physiological leakage proximal to the colon. This "true" catabolism seems to be slightly decreased or normal in most cases, independent of the clinical stage of the disease.

As to the question of colonic excretion of degradation products such as ^{131}I iodide and ^{131}I-amino acids, one experiment demonstrated this excretion to be insignificant. It is therefore highly probable that the non-protein-bound fraction of faecal activity is derived from bacterial decomposition of the labelled protein. The same proportions between protein-bound and non-protein-bound fractions were obtained by *Steinfeld* et al. (151).

That the leakage is exclusively localized to the diseased part of the intestine is evidenced by the fact that in one patient (case 19) with a permanent as well as a wet ileostomy there was no activity in the excretions of the former but in those of the latter.

It is also shown in IV (case 18) that more acute pathological conditions in the upper part of the intestine with increased catabolism of albumin are not necessarily

attended with increased faecal radioactivity when studied with ^{131}I-albumin.

Processes lower down, such as terminal ileitis, may in acute relapses cause an increase of the faecal content of radioactivity as a sign of pathological intestinal albumin leakage.

In quiescent phases of ulcerative colitis and terminal ileitis the intestinal leakage of albumin decreases and is sometimes quite normal, i.e. values < 1 % of the dose are found in the stools. Simultaneously the catabolism decreases to normal values.

After total colectomy with permanent ileostomy the intravascular albumin pool and the catabolic rate returned to normal. There was no radioactivity in the discharge of the permanent ileostomies after intravenous administration of ^{131}I albumin.

Studies with I albumin show an increased catabolic rate in intestinal disorders attended with leakage of albumin, but the faecal content of the label is dependent on the localization of the process, proximally or distally. In proximal processes reabsorption of the degradation products from the leaked-out I-albumin occurs distally. Such complete reabsorption is not possible when the disorder is in the distal part of the gut.

The acute radiation-syndrome

This syndrome is characterized by the early gastro-intestinal engagement with a simultaneous decrease of the serum albumin level. The possible connection between these two effects of radiation was first pointed out by Sullivan (154). H showed that, as regards the hypoalbuminaemia, total-body irradiation had the same effect as irradiation of the small intestine alone. An increased amount of ^{131}I PVP was found in the stools after irradiation (9 125).

A certain degree of intestinal paralysis after higher doses of irradiation is evident by the cause of the inability of these animals to pass faeces. Therefore, only small amounts of faecal radioactivity are regained. After lower doses, however some increase is seen.

In rabbits the hypoalbuminaemia caused by irradiation with 800r is statistically highly significant. The intravascular pool is not decreased to the same degree because of the plasma-volume increase. That this hypoalbuminaemia is not an effect of dilution is obvious from the preserved content of total protein.

The increase in degradation of albumin is very moderate in the 200—400r animals, while those irradiated with 800r (= LD 50) showed a more distinct rise of the fractional catabolic rate. After higher radiation doses no accurate calculations of the degradation rates could be made because of the prevailing non steady state.

Intestinal obstruction

As obstruction of the small intestine with concomitant disturbances in its function could possibly give rise to an elevated catabolism of albumin with hypoalbuminaemia, *the investigation presented in VII was performed. It* had been postulated earlier that a capillary damage may give rise to an increased leakage of plasma into the intestine

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That the leakage is exclusively localized to the diseased part *of the intestine* is evidenced by the fact that in one patient (case *19*) *with a permanent* as well as a wet ileostomy there was no activity in the excretions *of the former* but *in* those of the latter.

It is also shown in IV (*case 18*) that more acute pathological conditions in the upper part *of the* intestine with increased catabolism of albumin are not necessarily

decreased degradation. Furthermore, according to *Nasset* the regulating function of the endogenous proteins in the intestine will fairly soon be impaired during starvation and the supply of amino acids must be furnished from elsewhere. Here the muscle proteins appear as sources of amino acids (42). In cases where steady state is prevailing the synthesis is equal to catabolism, i.e. decreased. Apparently the state of the intravascular pool and the catabolic rate together determine the fractional and absolute synthetic rate.

The clinical part of the study revealed a certain increase of the catabolism during the first postoperative days after relief of the hindrance. Thereafter a normalisation or even a decrease occurred Whether this elevated catabolism is a continuation of an increase during the period of obstruction is not clear as investigations during that period are of course not justifiable. From the slight hypoalbuminaemia, arisen from 12—24 hours of simple obstruction with symptoms, it may be inferred that the catabolism is not elevated to any great extent. This is further verified in the experimental study where no increase was seen It was not possible to show any elevation of the normal leakage of albumin after 6—48 hours of simple obstruction neither above nor below the hindrance. These facts indicate that other mechanisms are active in the causation of hypoalbuminaemia

Strangulation with impairment of the venous return of blood entails quite different phenomena. A direct loss of blood and plasma into the damaged intestine occurs and may reach fairly high figures, such as $\sim$ 20 ml of plasma per hour in 1 m of strangulated intestine. However these losses do not reveal themselves by an increased catabolism. Here, too, there is a postoperative increase of catabolism. It can therefore be stated that intestinal leakage of albumin plays a negligible role in the hypoalbuminaemia occasionally present in intestinal obstruction. The problem of hypovolaemia and hypoalbuminaemia is due to other patho-physiological occurrences during the development of the ileus, such as intestinal distention and impairment of the peripheral as well as the central circulation These factors will be investigated further

High stenosis with starvation

In malnourished children a normal T/2 for ^{131}I albumin was observed by *Gitlin* et al (57) *Taylor* et al. (159) found no changes of intravascular albumin in volunteers starved 24 % of their body weights.

In paper IV two cases appear (cases 10 and 20) which show low or slightly subnormal catabolic rates (3 6—7 4 %) Both had stenosis of the cardia and had lost more than 25 % of their premorbid body weights because of malnutrition. Patient no 19 too, with a low body weight, partly because of malnutrition but without stenosis had a catabolic rate (7 2 %) close to the normal lower limit. In spite of the low catabolism, two of them (cases 10 and 19) had hypoalbuminaemia, while case 20 had a normal intravascular pool. Those with hypoalbuminaemia showed signs of extravascular retention of albumin while the total pools certainly were larger than may be expected from the intravascular values. — In a fourth case with cardiac stenosis and malnutrition, investigated later a low catabolic rate was found

Further data have since been published confirming this finding of low catabolism of albumin in malnutrition (34 78)
The mechanism behind this albumin-sparing regulation of the catabolism is not known However considering the gastrointestinal catabolism one explanation may be that the resting state of the intestine leads to a decrease in the blood flow As is pointed out in II this possibly means a lower leakage of albumin and a

The synthesis is fairly normal or even increased in ulcerative colitis, and in most cases the hypoalbuminaemia depends on loss of albumin int the large bowel. In cases with clinically recognizable cirrhosis of the liver as well as in cases with no detectable liver damage a defect synthesis may occur The same may be seen in patients with infectious complications. No conclusions as to the conformity between the capacity of albumin synthesis and the outcome of liver function tests can be drawn from this limited series. Malabsorption and malnutrition are probably not causative factors in the hypoalbuminaemia.

Whether a disturbance of the synthesis of albumin plays any part in the hypoalbuminaemia in the *acute adiatio yn drom* (VI) is difficult to tell. The decrease of the intravascular albumin pool is fairly rapid and plotting f the two curves as described above will only reveal that the synthesis is unable to compensate for the increased catabolism.

T what extent the liver cells are injured by the irradiation remains to be elucidated.

In ileus (VII) of 48 hours duration no decrease in the synthesis rate seemed to occur as the slope of the specific-activity curv was fairly constant. After the release of a strangulation there seems to be a more or less increased lbumin synthesis combined with decrease of catabolism within few days of the operation.

The indirect estimation of the albumin sy thesis in the liver presented in these papers is pproximatly since only rough figures are achieved for inability to balance or ability to overcompensate for the catabolism. The intravascular albumin pool may be maintained in two ways, namely by increased synthesis and/or substitution from the extravascular pool. To assess the significance of one of these two in relation to the other in non-steady state conditions is difficult (104) By using analogue computer it was possible to show that the synthesis plays a major role, at least in healthy animals (106)

In pathological conditions, however the circumstances may be quite different. From V as well as from other published observations, it can be deduced that the albumin synthesis in the healthy liver may be about doubled, but not more A liver diseased in some way will probably have lower capacity

What mechanism regulates the synthesis Disregarding the effects of certain hormones it may be postulated that any change in the catabolism or concentration of albumin initiates an equivalent change in the synthesis rate w thin reasonable limits. An example of this is the situation in cardiac stenosis, where the low catabolism is only compensated for as there is no hyperalbuminaemia in these cases. In tarvation and kwashiorkor the situation is the same the lowered catabolism is only compensated for

When the liver is diseased, as in cir rhosis where the synthesizing capacity is decreased, contrary explanation may be valid. It has been shown by several investigators that the catabolism of albumin is lowered (21 153) This is probably an effect and definitely not a cause of the decreased synthesis.

Synthesis of albumin

No methods for direct measurements of the synthesis of albumin are available. Only by using an indirect approach is it possible to achieve an approximate estimation of the synthesizing capacity of the liver

Plotting cpm/ml plasma on a semilogarithmic scale gives the fractional catabolic rate when the plasma volume is constant Plotting the specific activity (cpm/mg albumin) gives a slope which is an expression for the dilution of the labelled albumin made by newly synthesized albumin. A close fit between these curves means catabolism equal to synthesis, while a steeper slope of the latter curve indicates an increase of the synthesis, and vice versa. Such a deviation of the curves in either *direction infers per se a non steady state* which makes the quantitation approximative

Accordingly an increased fractional catabolic rate with a non-compensating synthesis means that sooner or later because of the induced hypoalbuminaemia, the absolute catabolic rate will decrease to a level where the synthesis is able to compensate and from then on a steady state will prevail

Thus it may be stated that in *gastric cancer* the synthesis is normal or decreased. *In IV it is said that the synthesis was* normal or slightly increased in 2 patients (cases 1 and 8) The proposal of a slight increase is uncertain as there was some denaturation of the labelled preparation (Amersham) which makes only relative comparison with the controls possible.

In patients nos. 11 and 12 with inoperable lesions but normal catabolic rates, there is a possibility that a *decreased* synthesis is the primary cause of the very slight hypoalbuminaemia.

In IV references are made to works on the nitrogen balance in gastric cancer These and the personal investigation indicate that malabsorption and malnutrition do not play any important part. The real cause of any lowered synthesis of albumin is unknown

In cases of *ulcerative colitis* the synthesis of albumin was generally normal or increased In some of those characterized *as normal it is very probable that there* was a slight increase but when referred to the total catabolism (g/day) instead of g/kg/day it seemed more correct to interpret it as normal The same observation was valid for the enteritis cases.

In one of the colitis and one of the enteritis cases only was there a decrease of the synthesizing capacity In the former (case 15) in a remitting phase, intestinal protein loss was not responsible for the low intravascular albumin *The only* explanation is a defect synthesis. The same was seen in case 24 where an infectious complication may have accounted for the decreasing influence on the synthesizing capacity of the liver

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In one of the colitis and one of the enteritis cases only was there a decrease of the synthesizing capacity In the former (case 15) in a remitting phase, intestinal protein loss was not responsible for the low intravascular albumin. The only explanation is a defect synthesis. The same was seen in case 24 where an infectious complication may have accounted for the decreasing influence on the synthesizing capacity of the liver

In VI an account is given of investigations into distribution and capillary transfer in *the radiation syndrome* which engages all organs. After irradiation with 800r or more a divergency between the plasma-activity and retained-dose curves appears. The exact interpretation of this finding is difficult, however as a considerable fraction of the radioactivity is found in the contents of the gastro-intestinal tract. An extravascular accumulation of albumin is not found except in the lungs.

In the irradiated rabbits there does not seem to be any increase of the total intravascular to extravascular transfer of albumin when measured by available methods. The circulatory condition of the animals was, however not considered here. It is quite possible that a lowered capillary pressure is in part responsible for the unchanged transfer rate. Later when the re-entry of the label begins and from then on, a steeper slope of the plasma activity curve appears. The connotation of this phenomenon may be a disturbed return of the label from the extravascular space to the blood. From the results it seems as if this disturbance were accelerated with increasing radiation doses.

A similar appearance of the plasma-activity curves, suggesting a disturbed re-entry of albumin, is seen in experimental *intestinal obstruction* (VII).

In these last two pathological conditions an accentuated non-steady state occurs, which makes certain calculations difficult. Thus, interpretation of results as to compartment sizes and even EV/IV ratios has been sparsely made partly because of the fact that a complete excretion of the degradation products of the ^{131}I albumin catabolism cannot at present be completely evidenced.

Analysis of the I-albumin content of tissues, referred to intravascular activity in animals with intestinal obstruction show that also the extravascular pools with a fast exchange, as in the parenchymatous organs, are deprived of their ^{131}I albumin to the same extent as the intravascular pool. In the gastro-intestinal tract there is a relative increase, indicating an accumulation on reference to the intravascular activity. More slowly exchanging pools, as those of the musculature, show a distinct increase relatively and, thus, constitute a greater part of the total-body activity.

As mentioned above, integration and differentiation of equations describing intercompartmental flow-rates and also compartment sizes can only be made in steady state. Under pathological conditions of more or less progressive or changing character no steady state prevails and, therefore only approximative calculations can be made.

Some findings are however indisputable such as the dissociation of the curves in gastric cancer indicating the presence of a greater extravascular pool with slow exchange. The wider the dissociation, the greater the extravascular pool in relation to the intravascular one. Only in this disease was a dissociation of such a degree observed. In rabbits irradiated with doses > LD 50, a dissociation was also seen. However the retention of activity seems here to consist partly of degradation products. Even in intestinal obstruction it seems that such a disturbance is induced, although circulatory factors are also involved. At the present time it is not pos-

CHAPTER IX

Distribution and capillary transfer of albumin

Even under normal conditions there exist problems in obtaining an exact picture of the extravascular *compartments* and their sizes. These problems are still greater under pathological conditions with non steady state and more or less accelerated catabolism.

It is doubtful (32) whether a uniform specific activity is obtained in cases with an increased catabolism. This means that *no method* assuming homogeneous specific activity may be used. Thus, only the equilibrium time method and the compartment analysis remain.

In papers IV—VII the equilibrium-time method has generally been used. No values for the extravascular pool have been given when non-steady state prevails, as such *figures would* only reflect the situation at a given moment.

In IV on *gastric cancer* is discussed a certain finding expressed as the quotient K_{iv}/K_{rt} where the K_{iv} is synonymous with λ in the other papers, and K_{rt} is λ for the total-body activity. This quotient expresses the dissociation between the slopes *of the plasma activity and total* body-activity curves. On comparison with the K_{iv}/K_{rt} of the controls, there are differences in that the values are high for the inoperable cancer cases, especially those with ascites.

The increase *of* this quotient indicates a continuous extravascular accumulation of radioactivity (^{131}I-albumin). As to the real size of this or these extravascular pools, nothing can be *concluded* as parallellity i.e. steady state, was never achieved during the period of investigation. In the three *postoperatively investigated* cases normal or nearly normal values of K_{iv}/K_{rt} were obtained.

In *ulcerative colitis* no such tendency of the K_{iv}/K_{rt} was seen. In all except 4 cases normal figures were obtained. One was case 4 with a normal catabolic rate. This patient with a deteriorating general *condition* had undergone steroid therapy for some time. At operation an oedematous dilated bowel with mucosal gangrene was removed. The walls contained 4.3 % of the dose administered. Whether this is a pathological *figure is impossible* to tell. The explanation of the raised quotient in the other three cases is obscure. In 2 out of the 11 patients with colitis where the extravascular albumin mass could be estimated with any accuracy the extravascular pool was smaller than the intravascular. In the other 9 cases the EV/IV ratio varied between 1.11 and 1.54 except in one case where it was 2.14. Generally there was a *fairly normal distribution*. Case *6 with* a catabolism of 20.8 % had an EV/IV *ratio* of 0.62, *while case 18* with acute enteritis and a catabolism as high as 23.8 % did not show an *inverse EV/IV ratio*.

Plasma volumes and intravascular albumin under pathological conditions

Plasma-volume

Generally plasma-volume (PV) red-cell volume (RCV) blood-volume (TBV) and intravascular albumin are accounted for as referred to body-weight. This way is approvable under normal conditions, when the body-weight is a stable parameter. Ideal body-weight and body surface have been suggested as suitable parameters, too.

In conditions where the body-weight is subjected to more or less extensive variations, reference to body-length has been suggested (55, 128, 146, 180).

In IV and V comparisons are made between TBV, PV and RCV correlated to body-weight and body-length in cases of gastric cancer and of ulcerative colitis. It is shown that reference to body-weight may give significantly increased values for PV, for instance in patients with inoperable gastric carcinoma or ulcerative colitis, acute as well as chronic. When referred to body-length, on the other hand, quite normal figures for PV are obtained.

Papers VI and VII deal with changes of plasma-volumes in the radiation syndrome and intestinal obstruction. After irradiation of rabbits with ≥ 800r it is shown that the plasma-volume is preserved and even increased by up to 20—30 %. No differences between measurements peripherally or centrally were obtained. In simple intestinal obstruction a distinct decrease of PV is found in dogs after only 24 hours, not so in man however.

It is postulated that an expansion of the plasma-volume occurs in some diseases, for instance ulcerative colitis (151) and even that this expansion and the hypoalbuminaemia would depend on a dilution. Here evidently the variations in body-weight are not taken into consideration. As seen above, correlation to body-weight gives an impression of increased PV in conditions attended with weight loss, such as gastric cancer, ulcerative colitis, and cardiac stenosis with starvation. If body-length is used as reference, it is obvious that the PV is unaltered.

It is therefore suggested that body-length is the parameter of choice when accounting for PV as well as TBV and RCV in cases with inconstant body-weights.

The increase of PV after irradiation is at the present time difficult to explain. *Lukis* (100) and *Lukin & Gregersen* (101) made the same observation in irradiated dogs, but also registered a marked decrease just prior to death. No such decrease was found by us in those rabbits that died a few hours after the investigation. This increase of PV is partly com-

sible to make any quantitative calculations as this would involve very uncertain assumptions.

From the investigations in III, VI and VII it may be postulated that what is at present called "increased capillary permeability" to proteins in certain pathological conditions is also a matter of "decreased return of proteins to the intravascular space". In an earlier chapter it was shown that the normal capillary transfer of proteins (albumin) is much greater than what is returned via the lymphatic route (determined by current methods of measuring lymph flow). It was made probable that also a transcapillary return of proteins to the circulation occurs.

It is thus not improbable that under some pathological conditions characterized by "increased capillary permeability" disturbances in this process may also play a part.

These investigations into capillary permeability in pathological conditions have opened up new aspects of the capillary transfer. As regards methodology as well as the interpretation of the results, many questions remain to be solved.

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sible to make any quantitative calculations, as this would involve very uncertain assumptions.

From the investigations in III VI and VII it may be postulated that what is at present called "increased capillary permeability" to proteins in certain pathological conditions is also a matter of "decreased return of proteins to the intravascular space" In an earlier chapter it was shown that the normal capillary transfer of proteins (albumin) is much greater than what is returned via the lymphatic route (determined by current methods of measuring lymph flow) It was made probable that also a transcapillary return of proteins to the circulation occurs.

It is thus not improbable that under some pathological conditions characterized by increased capillary permeability disturbances in this process may also play a part.

These investigations into capillary permeability in pathological conditions have opened up new aspects of the capillary transfer As regards methodology as well as the interpretation of the results, many questions remain to be solved.

Therapeutic aspects

In view of the results of the investigations presented above, and considering the different functions of albumin, it seems adequate to maintain the intravascular albumin pool as normal as possible even in pathological conditions.

Therapeutic measures against disturbances in serum albumin metabolism serve three main purposes

1 Replacement in hypoalbuminaemia with lowered plasma-volume or blood-volume, mostly seen in certain acute pathological conditions.

2. Correction of hypoalbuminaemia when the plasma-volume is preserved.

3. Removal of factors causing the deficiencies, either by surgery or by medical measures.

As seen from IV V and VI determinations of total protein in plasma most often yield normal values and do not reveal hypoalbuminaemic states. To achieve true picture of the serum-protein situation it is necessary to perform electrophoresis and sometimes plasma volume determinations.

Treatment according to 1 will be indicated in some acute conditions, such as intestinal obstruction, particularly with strangulation. The problem is not only to restore proteinaemic deficiency but also to resutitute plasma-volume and/or blood-volume the sooner the better In surgical cases this measure should be started preoperatively and continued during and after operation. These aspects are well covered by the general rules for shock therapy where blood, plasma, and albumin are the main constituents.

In hypoalbuminaemic subjects with maintained plasma-volumes, the cause of the deficiency has to be considered. Chronic or more acute losses, insufficient synthesis for different reasons, or pathological distribution are the causes mostly met with. Often a combination of some or all of them will appear

In diseases with continuous losses and with decreased or normal synthesis, as for instance in gastric cancer and some cases of ulcerative colitis, it is advisable to administer plasma or albumin. It does not seem justifiable to rely upon the synthesis to compensate for increased catabolism or losses, and therefore, providing for a sufficient supply of amino acids may not be enough.

This is valid also in cases where the question of surgery will not arise, viz. certain cases of ulcerative colitis, and cases of gastric cancer with ascites, where laparocentesis is repeatedly performed. In the latter cases there is a more or less pronounced continuous loss of intravascular albumin into the peritoneal cavity and no spontaneous extravascular supply can ever compensate for these internal losses the intravascular albumin pool has to be replaced by transfusions of plasma and albumin.

pensatory to the evidently diminished RCV but the mechanism is unclear

Obviously quite different pathophysiological mechanisms are engaged in intestinal obstruction where a true decrease of PV occurs. The duration of obstruction and the degree of distension seem to play an important role although the exact mechanism is obscure.

Intravascular albumin

Generally the intravascular albumin pool is accounted for either as a total or as g/kg of body weight. In gastric cancer and acute ulcerative colitis no or small decreases are found in relation to body-weight while reference to body length does give significantly diminished figures. In cardiac stenosis with starvation, but without hypoalbuminaemia an impression of hyperalbuminaemia is obtained when reference to weight is made. If the body length is used instead, the true state i.e. normoalbuminaemia, is revealed.

It is therefore also suggested that the stable parameter of body-length be used when accounting for intravascular albumin mass in patients with weight loss otherwise a false impression of normoalbuminaemia will be obtained.

In the acute radiation-syndrome the relative hypoalbuminaemia is not matched by a corresponding lowering of the intravascular albumin pool, because of the increase of PV The opposite is seen in intestinal obstruction, where a relative hypoalbuminaemia only partly tells the true decrease of the intravascular albumin pool, because of the diminished PV

In certain conditions where PV may fluctuate in one direction or the other the relative albumin content in plasma does not reveal the true deficit.

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In diseases such as ulcerative colitis and Crohn's disease without superimposed infections in which the synthesis of albumin in the liver may increase, amino acids albumin, and plasma may be given to normalize the serum albumin level. It must be kept in mind however that accumulated evidence indicates that the synthesis capacity of the healthy liver can only increase two-fold.

The third purpose, to remove the pathological process itself sometimes necessitates surgical intervention. In such cases it is mandatory to obtain a serum-protein pattern as normal as possible preoperatively. Out of clinical experience it is well known that hypoalbuminaemia in patients subjected to surgery will often lead to more or less severe complications. Imperfect or delayed healing of anastomoses in gastro-intestinal surgery or insufficiency of sutured wounds in general may give rise to peritonitis, abscesses, and fistulas. Such complications are often deleterious. A preoperative replacement of the intravascular albumin pool must therefore be aimed at simultaneously with restoration of RCV and total haemoglobin when necessary. In elderly patients with hypoalbuminaemia, intense preoperative replacement therapy of that kind may entail certain circulatory risks. It may therefore be advisable not to pursue full restitution preoperatively but to extend the replacement over the per- and postoperative periods also.

Another fact which should not be overlooked is that in patients with hypoalbuminaemia and preserved plasma volumes, some of the homeostatic compensatory "reserve power" normally available has already been used up. When it comes to surgery, less of this "reserve power" will remain, which may lead to more or less acute circulatory and metabolic failures.

General Summary and Conclusions

This supplement and papers I—VII (listed on page 7) deal with problems concerning the metabolism and distribution of serum albumin under normal and some pathological conditions.

Part I

In this part is given a short survey on the different functions of albumin. Investigations by others are presented demonstrating the relevance of using iodine-labelled albumin for metabolic studies. The background and aim of the investigation are described.

The methods used in the preparation and labelling of albumin are briefly accounted for.

No differences between labelled and unlabelled albumin were revealed by ultracentrifugation (III) or by immunological tests. Also metabolically the preparations proved satisfactory.

The methods employed for measurement of samples and plasma-volume determinations are reviewed.

The procedures used in the clinical as well as the experimental determinations of the gastro-intestinal albumin leakage are discussed.

Part II

This part and papers I—III deal with the normal metabolism.

Chapter I Here a survey is given of different ways of calculating the catabolic rate. In the present control cases good agreement was obtained between the methods where reference was made to the intravascular pool. A good conformity was found between the results by investigators who referred catabolic rate to the intravascular pool.

Investigations into the catabolism of albumin in dogs (III) and rabbits (VI) are accounted for. There was an equally good agreement as in man between the methods used.

Chapter II describes the results of the quantitative investigations into the gastro-intestinal leakage of albumin in man (I) and in dogs (II). Somewhat different methods were used in calculations in the two species. In man, quantitative determinations of the leakage into the stomach and the upper part of the jejunum were performed. In dogs it was shown that albumin leaked into the entire small intestine. A maximal leakage per unit length was found in the duodenum. In the jejunum (proximal two-fifths) and in the ileum (distal three-fifths) a gradual decrease of the leakage occurred in the distal direction. By reference to the daily catabolism the small intestine, including the duodenum, was found to be responsible for the main part of it (~ 5/6) while the gastric part was [illegible]. It is proposed that the leakage is directly correlated to the blood-flow to the respective parts of the alimentary canal.

Chapter III elucidates the irrelevancy of using ^{51}Cr-albumin for calculations of the normal physiological leakage of albumin. ^{51}Cr-albumin was shown to be heteroge

neous product. Initially it disappeared rapidly from the blood and also continuously the elimination was accelerated. A high uptake by the liver and the lymph glands was registered. The jejunal leakage of ^{51}Cr albumin was smaller than that of ^{131}I albumin given simultaneously. No ^{51}Cr albumin leaked into the stomach.

Chapter IV. It has been convincingly shown by others that the breakdown of albumin is a first order process. From personal as well as other investigators' results on catabolic rates relatively and absolutely in various mammal species, it could be deduced that the degradation process is a "true" metabolic one following the "surface law", i.e. the daily amount catabolized is correlated to the body-mass raised to the power of 0.684. This entails the fact that a constant amount of 7 g as a mean is broken down per sq m body-surface area, independent of body-weights and species. As similarly good, though approximative correlation between plane intestinal surface area and body-surface area was demonstrated, the leakage of albumin per sq cm intestine is possibly uniform throughout the mammal species. A conformity is shown to exist between man and dog. The purpose of this process is discussed.

Chapter V gives a brief discussion of the limited possibilities of determining the albumin synthesizing capacity of the liver.

Chapter VI. The different methods for calculating the distribution of albumin in various extravascular compartments are briefly reviewed (III). It was shown that there are methodological as well as individual differences. The number of exponentials that can be derived from plasma activity curves may vary both between and within the species. This variation seems to be dependent upon the sampling intervals as well as the way in which the curves are analyzed.

The presence of a slowly exchanging extravascular compartment with "delayed steady state" is shown in man.

By the *double-isotope technique* the specific amount of extravascular albumin in different organs and organ systems was determined in dogs. The limitations of this method are briefly discussed.

The *total* capillary transfer rates were determined empirically and experimentally. The results of the empirical determinations were compared with those obtained by others. For the experimental determinations it was assumed that, as long as the elimination curve was straight, the re-entry of the label into the circulation was negligible. There were obvious differences between the results with the two methods. Higher values for the initial disappearance rates were obtained experimentally, the higher the smaller the species investigated. That no denaturation of the protein was responsible for this was shown by the use of a "screened" preparation of I albumin. Assuming a constant intravascular albumin pool (per kg of body-weight), the transfer rates obtained gave a total capillary transfer which was significantly correlated to the body-mass raised to the power of 0.829, i.e. a constant amount averaging 96 g is transferred per sq m body-surface area, independent of body-weights and species ("surface law"). The analogy of this with oxygen consumption and cardiac output is pointed out. The relation to capillary surface area and blood flow are discussed.

The discrepancy between this capillary

transfer and available data on the lymphatic protein return from a quantitative viewpoint is discussed.

Part III

This part and papers IV—VII deal with the metabolism of albumin under some pathological conditions engaging the gastro-intestinal tract gastric cancer ulcerative colitis, terminal ileitis, the acute radiation-syndrome and intestinal obstruction.

Ch pter VII An account is given of the catabolism of albumin in these conditions. In *gastric cancer* an increased leakage of albumin was shown to occur in achlorhydric cases, even in comparison with achlorhydric cases without cancer This elevated gastric albumin loss could some times be responsible for the whole increase of the catabolic rate, which never reached extreme values. In normo-or hypochylic cases it was not possible to state with certainty whether an increased leakage was responsible for the hypoalbuminaemia. N correlation between the histological type of the tumour and the leakage was found in the investigated cases.

Ul erat olitis was nearly always attended with hypoalbuminaemia. This was in most cases caused by loss of albumin through the diseased wall of the large bowel The losses were greatest in acute relapses where a more than two-fold increase of the catabolic rate could be seen. It could be demonstrated that the losses were wholly colonic. Exclusion of the faecal losses of I albumin in calculations of catabolism gave subnormal-normal values. After total colectomy the catabolic rate and the intravascular pool returned to normal. Remitting cases also revealed normal catabolic rates and no faecal losses.

Lesions in the small intestine (acute jejunoileitis) did not necessarily entail faecal losses, because of reabsorption of the degradation products the losses into the intestine were mirrored in a true increase of the catabolism.

The *acute radiation synd ome* was investigated experimentally in rabbits. An increase of the catabolic rate was found up to LD 50 as a maximum about twice the normal value. It could be demonstrated that intestinal losses were responsible for this. After higher radiation doses a non-steady state made calculations impossible, partly because of a disturbed reabsorption of the degradation products of the [131]I albumin from the intestine.

In simple *intestinal obstruction* a very moderate increase of the catabolic rate was found after release of the obstruction. In a case of paralytic ileus with enteritis, the reabsorption was obviously lowered as faecal radioactivity was high. Experimentally no increase of catabolism was found, nor was the intestinal leakage of albumin increased In strangulation, heavy losses of plasma into the diseased intestine were noted.

Cardiac stenosis with malnutrition was attended with a lowered catabolism. The possible reasons for this are discussed.

Chapter VIII The difficulties in calculating the synthesis in non-steady state are obvious. On certain presumptions it may be approximately estimated whether or not the synthesis is able to compensate for an increased catabolism. In *gastric cancer* the synthesis was found to be normal or decreased. In *ulcerative colitis* it seemed mostly to be normal or increased. In some

chronic cases with liver damage, concealed or not, an insufficient albumin synthesis may be the cause of persistent hypoalbuminaemia. Malabsorption did not seem to play a role In one case of acute enteritis with a two-fold increase of the absolute catabolic rate an equivalent increase of the synthesis could be deduced. No conclusions could be drawn as to the albumin production after *total body irradiation*. In *intestinal obstruction* or at least after release of the hindrance a normal or even slightly increased synthesis seemed probable

Some aspects of the interrelation of synthesis-catabolism are briefly discussed.

Chapter IX In conditions characterized by non-steady state no definite conclusions can be drawn concerning the distribution of albumin. In cases with *gastric cancer* inoperable without or with ascites, divergency between the plasma-activity curves and the retained dose curves was found. This phenomenon seemed indicative of an accumulation extravascularly or rather a very much delayed intra extravascular equilibrium. Generally no such disturbance was seen in *ulcerative colitis* On the contrary in this disease a decreased extravascular activity was sometimes found After *irradiation* with high doses (> LD 50) an accentuated non-steady state was observed with a relative increase of extravascular activity

In *intestinal obstruction* a relative increase of extravascular activity occurred particularly in the muscles and the intestinal wall. No quantitative interpretations could be made because of the prevailing non-steady state

Investigations into total capillary transfer at different intervals after irradiation showed unchanged initial disappearance rate. This is suggestive of an unchanged total capillary transfer as is the steeper subsequent slope of the curves indicative of a disturbed re-entry of the protein into the circulation. This disturbance may also be valid in intestinal obstruction. The limitations of the method the results, and their physiological probability are discussed

Chapter X In pathological conditions, such as some of those investigated gastric cancer ulcerative colitis, and cardiac stenosis, the plasma volumes were preserved irrespective of the degree of hypoalbuminaemia. Very often these diseases are attended with variations in body weight a fact which makes the body weight unsuitable as reference parameter for plasma volumes as well as for intravascular albumin. It is shown that body length being a stable parameter is the reference of choice

In *chapter XI* are discussed the therapeutic measures necessary to compensate for the hypoalbuminaemia, with respect to losses, increased catabolism, and disturbances in the production of albumin Surgical aspects of the replacement therapy in subjects who will undergo operative treatment are discussed

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References

1 *Abdou I & Tarver H.* Plasma proteins II. Relationship between circulating and tissue protein. J biol. Chem. *190* 781 1951

2. *Adolph E. F.* Quantitative relations in physiological constitutions of mammals. Science *109* 579 1949

3 *Albright F F., Bartter C & Forbes A. P.* The fate of human serum albumin administered intravenously to a patient with idiopathic hypoalbuminaemia. Trans. Ass. Amer Phycns *62* 204 1949

4 *Areskog, N H., Arturson G., Grotte G & Wallenius G.* Studies on heart lymph. Acta Dis. Child. *39* 182, 1964

5 *Armstrong S H Jr., Mc Leod K., Wolter J & Kukral J.* Persistence in blood of radioactive label of albumin, gammaglobulins, globulins of intermediate mobility studied with S^{35} and paper electrophoresis Methods and preliminary results. J Lab. clin. Med. *43* 918, 1954

6. *Armstrong, S H Jr., Kukral J Hershman J., Mc Leod K. Wolter J & Bronsky D* Persistence in blood of radioactive label of albumins, gammaglobulins and globulins of intermediate mobility comparisons of gammaglobulins labeled with S^{35} and I in the same subject. J Lab. clin. Med. *45* 51 1955

7 *Baker C H* Fibrinogen-I^{131} T 1824 and red cell-Cr^{51} spaces following haemorrhage Amer J Physiol. *205* 527 1963

8. *Baker C H & Wycoff H D* Time concentration curves and dilution spaces of T 1824 and I labeled proteins in dogs Amer J Physiol. *201* 1159 1961

9 *Barandun S., Aebersold J Bianchi, R., Kluthe R., v Muralt G Poretti, G & Riva, G.* "Proteindiarrhoe Schweiz. med. Wschr *90* 1458, 1960

10. *Berson A., Rotschild, M A., Yalow R, S & Berson S.* Distribution and metabolism of I^{131} labeled human serum albumin in congestive heart failure with and without proteinaemia. J clin. Invest. *34* 1359 1955

11 *Becker, W L., Volwiler W., Goldsworthy P D., Gerby L E., Reynolds W E., Stogsdill R. & Stemler R. S.* Studies of I -albumin catabolism and distribution in normal young male adults. J clin. Invest. *41* 1312, 1962

12. *Bennett, H S., Luft J H & Hampton, J C.* Morphological classification of vertebrate blood capillaries. Amer J Physiol. *195* 381 1959

13. *Bennhold H.* Überblick über die Transportfunktion der Serumeiweisskörper Proteides of the biological fluids. IX Colloquium. Elsevier Publ. Co Amsterdam. 1961

14 *Bennhold H.* Das menschliche Serumalbumin. Bull. schweiz. Akad. med. Wiss. *1* 62, 1961

15 *Bennhold H & Kallee E.* Comparative studies on the half-life of I^{131} labelled albumins and non-radioactive human serum albumin in a case of analbuminaemia. J clin. Invest. *38* 863 1959

16. *Bergner P E.* The significance of certain tracer kinetical methods especially with respect to the tracer dynamic definition of metabolic turnover Acta radiol. Suppl. 10, 1962

17 *Berman, M & Schoenfeld R.* Invariants in experimental data on linear kinetics and the formulation of models. J appl Phys. 2 1361 1956

18 *Berson, S Yalow R S Schreiber S & Post J.* Tracer experiments with ^{131}I-labelled human serum albumin distribution and degradation studies. J clin. Invest. 32 746, 1953.

19. *Birke G Liljedahl, S-O & Plantin, L-O* Albuminomsättningen vid stora brännskador studerad medelst J^{131}-märkt albumin. Nord. Med. 60 963, 1958.

20. *Birke G Liljedahl S-O Plantin L-O & Wetterfors, J* Role of the stomach in the metabolism of albumin. Nord. Med. 62 1741 1959

21 *Birke G O Uberg, R., Liljedahl S-O Olhagen R., Plantin L.-O & Wetterfors J* Albumin Pathophysiological and clinical aspects. Nord. Med. 65 813 1961

22 *Blalock, A Robinson, C S Cunningham R S & Gray M E* Experimental studies on lymphatic blockage. Arch. Surg. 34 1049 1937

23 *Bloch Edm. & Cogan S J* The transit of large molecules and particulate matter across individual endothelial cells, analyzed in living animals with television microphotometry Angiology 15 353 1964

24 *Boberg, J* Personal communication 1964

25 *Brody S* Bioenergetics and Growth. Reinhold Publ Corp New York. 1945

26 *Brown R & Danielli, J F* Active transport and secretion Academic Press New York 1954

27 *Bittiger L. E & Carlson, L. A.* Electrophoretic studies on serum glucoproteins Clin. Chem. Acta 5 91., 1960

28 *Campbell R M Cuthbertson D P Matthews C M & McFarlane A S* Behaviour of ^{14}C- and ^{131}I-labelled plasma proteins in the rat Int. J appl Radiat 1 66, 1956

29 Campbell R M Cuthbertson, D P *Mackie W M Farlane A S Phillipson A T & Sudsaneh S* Passage of plasma albumin into the intestine of the sheep. J Physiol. (Lond.) 158 113, 1961

30 *Chapman A., Jeffries G H & Sleisenger M H* The measurement of enteric plasma protein loss. An evaluation of the I^{131}-labelled albumin — oral Amberlite resin technique. Symposium on physiology and pathophysiology of plasma protein metabolism. Grindelwald 1964 Hans Huber Verlag, Stuttgart. In print.

31 *Citrin, Y Sterling, K. & Halsted J A.* The mechanism of hypoproteinaemia associated with giant hypertrophy of the gastric mucosa. New Engl. J Med. 257 906, 1957

32. *Cohen, S Freeman, T & McFarlane A S* Metabolism of ^{131}I labelled human albumin. Clin. Sci. 20 166, 1961

33. *Cohen S & Gordon, A. H* Catabolism of plasma albumin by the perfused rat liver Biochem. J 70 A4 1958.

34 *Cohen, S & Hansen J D* Metabolism of albumin and γ-globulin in kwashiorkor Clin. Sci. 23 351 1962.

35 *Cohen S Holloway R. C Matthews, C M & M Farlane, A. S.:* Distribution and elimination of ^{131}I and ^{14}C-labelled plasma proteins in the rabbit. Biochem. J 62 143 1956.

36 *Cohen, S & Schamroth J* Metabolism of ^{131}I-labelled albumin in African subjects. Brit. med. J 1 1591 1958.

37 *Cruickshank, E. W & Whitfield J C.* The behaviour of T 1824 in circulating blood and modified method for the estimation of plasma-volume. J Physiol. (Lond.) 104 52, 1945

38 *Dewey W C* Vascular — extravascular exchange of I^{131}-plasma proteins in the rat. Amer J Physiol. 197 423, 1959

39 *Dixon, F J & Maurer P H* Effects of large infusions of heterologous serum proteins on serum protein metabolism of rabbits. J exp. Med. 101 233 1955

40. *Dixon, F J & Weigle W O.:* The

References

1 *Abdou I & Tarver H* Plasma proteins II. Relationship between circulating and tissue protein. J biol. Chem. *190* 781 1951

2. *Adolph E F* Quantitative relations in physiological constitutions of mammals. Science *109* 579 1949

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5 *Armstrong, S H Jr Mc Leod K Wolter J & Kukral J* Persistence in blood of radioactive label of albumin, gammaglobulins, globulins of intermediate mobility studied with S^{35} and paper electrophoresis Methods and preliminary results. J Lab. clin. Med. *43* 918, 1954

6. *Armstrong, S H Jr Kukral J Hershman J Mc Leod K. Wolter J & Bronsky D* Persistence in blood of radioactive label of albumins, gammaglobulins and globulins of intermediate mobility: comparisons of gammaglobulins labeled with S^{35} and I^{131} in the same subject. *J Lab.* clin. Med. *45* 51 1955

7 *Baker C H* Fibrinogen I^{131} *T 1824* and red cell-Cr^{51} spaces following haemorrhage. Amer J Physiol. *205* 527 1963

8 *Baker C H & Wycoff H D* Time concentration curves and dilution spaces of T 1824 and I^{131}-labeled proteins in dogs. Amer J Physiol. *201* 1159 1961

9 *Barandun S Aebersold J Bianchi R Kluthe R., v Muralt G Poretti G & Riva G* "Proteindiarrhoe" Schweiz. med. Wschr *90* 1458, 1960

10. *Bauman A., Rotschild M A., Yalow R S & Berson S* Distribution and metabolism of I^{131} labeled human serum albumin in congestive heart failure with and without proteinaemia. J clin. Invest. *34* 1359 1955

11 *Beeken, W L Volwiler W Goldsworthy P D Garby L E Reynolds W E., Stogsdill R. & Stemler R S* Studies of I^{131}-albumin catabolism and distribution in normal young male adults. J clin. *Invest. 41* 1312, 1962.

12 *Bennett H S Luft J H & Hampton J C* Morphological classifications of vertebrate blood capillaries. Amer J Physiol. *196* 381 1959

13 *Bennhold H* Überblick über die Transportfunktion der Serumeiweisskörper Protides of the biological fluids. IX Colloquium. Elsevier Publ. Co. Amsterdam. 1961

14 *Bennhold H* Das menschliche Serumalbumin. Bull. schweiz. Akad. med Wiss. *17* 62, 1961

15 *Bennhold H & Kallee E* Comparative studies on the half life of I^{131} labelled albumins and non-radioactive human serum albumin in a case of analbuminaemia. J clin. Invest *38* 863 1959

16. *Bergner P E* The significance of certain tracer kinetical methods, especially with respect to the tracer dynamic definition of metabolic turnover. Acta radiol. Suppl. 210, 1962

17 *Berman M & Schoenfeld R* Invariants in experimental data on linear kinetics and the formulation of models. J appl. Phys. *27* 1361 1956

18 *Berson S Yalow R. S Schreiber S & Post J* Tracer experiments with I-labelled human serum albumin distribution and degradation studies. J clin. Invest. *32* 746, 1953.

19 *Bark G Liljedahl, S-O & Plantin, L-O* Albuminomsättningen vid stora brännskador studerad medelst J -märkt albumin. Nord. Med. *60* 963, 1958.

20 *Birke G Liljedahl, S-O Plantin L-O & Wetterfors J* Role of the stomach in the metabolism of albumin. Nord. Med. *62* 1741 1959

21 *Birke G Gullberg, R., Liljedahl S-O Olhagen, R., Plantin, L-O & Wetterfors J* Albumin. Pathophysiological and clinical aspects. Nord. Med. *65* 613 1961

22 *Blalock, A Robinson C S Cunningham R S & Gray M E* Experimental studies on lymphatic blockage. Arch. Surg. *34* 1049 1937

23. *Bloch, Edw. & Cozas S I* The transit of large molecules and particulate matter across individual endothelial cells, analysed in living animals with television microphotometry Angiology *15* 353 1964

24 *Boberg, J* Personal communication 1964.

25 *Brody S* Bioenergetics and Growth. Reinhold Publ Corp. New York. 1945

26 *Brown R & Danielli, J F* Activ transport and secretion. Academic Press. New York 1954

27 *Böttiger L. E. & Carlson L. A.* Electrophoretic studies on serum glucoproteins Clin. hum Acta 5 912, 1960.

28 *Campbell R M Cuthbertson, D P Matthews C M & McFarlane A S* Behaviour of [14]C- and [131]I labelled plasma proteins in the rat. Int. J ppl Radiat. *1* 66, 1956

29 *Campbell R M Cuthbertson, D P Mackie W M Farlane A S Phillipson, A. T & Sudsaneh, S* Passage of plasma albumin into the intestine of the sheep. J Physiol. (Lond.) *158* 113 1961

30. *Chapman A., Jeffries G H & Sleisenger M H* The measurement of enteric plasma protein lost. An evaluation of the I[131] -labelled albumin — oral Amberlite resin technique. Symposium on physiology and pathophysiology of plasma protein metabolism. Grindelwald 1964. Hans Huber Verlag, Stuttgart. In print.

31 *Citrin, Y Sterling, K. & Halsted, J A* The mechanism of hypoproteinaemia associated with giant hypertrophy of the gastric mucosa. New Engl. J Med. 257 906, 1957

32. *Cohen, S Freeman, T & McFarlane A. S.:* Metabolism of [131] I-labelled human albumin. Clin. Sci. *20* 166, 1961

33. *Cohen, S & Gordon A. H* Catabolism of plasma albumin by the perfused rat liver. Biochem. J *70* 544, 1958.

34 *Cohen, S & Hansen, J D.:* Metabolism of albumin and γ-globulin in kwashiorkor Clin. Sci. *23* 351, 1962.

35 *Cohen S Holloway R. C Matthews, C M & McFarlane A. S* Distribution and limitation of [131]I and [14]C-labelled plasma proteins in the rabbit. Biochem. J 62 143 1956.

36. *Cohen, S & Schamroth, J.:* Metabolism of [131]I-labelled albumin in African subjects. Brit. med. J *1* 1391 1958.

37 *Cruickshank, E. W & Whitfield, J C.* The behaviour of T 1824 in circulating blood and modified method for the estimation of plasma-volume. J Physiol. (Lond.) *104* 52, 1945

38. *Dewey W C* Vascular — extravascular exchange of I [131]-plasma proteins in the rat. Amer J Physiol. *197* 423 1959

39 *Dixon F J & Maurer P H* Effects of large infusions of heterologous serum proteins on serum protein metabolism of rabbits. J exp. Med. *101* 233 1955.

40. *Dixon, F J & Weigle W O* The

relationship of the rates of *serum* protein metabolism heterologous serum protein catabolism and the time and magnitude of the antibody response. Ann. N Y Acad Sci. *70* 69 1957

41 *Dreisbach L. & Nasset E. S.*. Absorption of carbohydrate and protein as affected by feeding cornstarch, banana or glucose. J Nutr *53* 523 1954

42. *Elman R.* Parenteral alimentation in surgery Paul Hoeber Inc. New York. 1947

43 *Engström N., Ljungqvist A Persson B & Wetterfors J.*. Tuberous sclerosis with a localized angiomatous malformation in the ileum and excessive albumin loss into the lower intestinal tract. Pediatrics. *30* 681 1962

44. *Fine J & Seligman A M* Traumatic shock. VII A study of the problem of the lost plasma in haemorraghic tourniquet and burn shock by the use of radioactive iodo-plasma protein. J clin. Invest *23* 720. 1944.

45 *Fisher R B & Parsons D S* The gradient of mucosal surface area in the small intestine of the rat. J Anat. (Lond.) *84* 272 1950.

46. *Franks J J Edwards K W Lackey W W & Fitzgerald J B* The role of the gut in the albumin catabolism II Studies in enterectomized rabbits. J gen. Physiol. *46* 427 1963

47 *Franks J J Mosser E. L. & Anstadt G L* The role of the gut in albumin catabolism I Studies in the jejunoileoectomized rabbits. J gen. Physiol. *46* 415 1963

48. *Freeman W L.* Lymphatic path ays from the intestine in the dog. Anat Rec. *82* 543 1942

49 *Freeman T & Gordon, A H* Metabolism of albumin and gammaglobulin in protein *deficient rats. Clin.* Sci. *26* 17 1964

50 *Freeman T., Gordon A H & Humphrey J H* Distinction between catabolism of native and denaturated proteins by the isolated perfused liver after carbon loading Brit J exp. Path. *39* 459 1958.

51 *Freeman T & Matthews C M Mc Farlane A S Bennhold H & Kallee E* Albumin labelled with Iodine 131 in an analbuminaemic subject. Nature. *183* 606, 1959

52. *Frenkel N & Ingbar S H* Concentration gradients for inorganic I 131 and chloride in mixed human saliva. J clin. Invest *32* 1077 1953

53 *Friedberg, W* Relation between pool size and catabolism of albumin in the mouse. *Amer J Physiol. 204 501* 1963

54 *Friedberg, W Walter H & Haurowitz., F* Fate in rats of heterologous protein labelled internally by S^{35} and externally by I 131 Science. *121* 871 1955

55 *Gibson J Jr & Evans W Jr* Clinical studies of blood volume, relation *of* plasma and total blood volume to venous pressure blood velocity rate physical *measurements*, age *and sex* in 90 normal humans J clin. Invest. *16 317 1937*

56. *Gitlin D* Distribution dynamics of circulating and extravascular I 131 plasma proteins. An. N Y Acad Sci. *70* 122 1957

57 *Gitlin D Cravi to J Frenk S Montano E L., Galvan R R Gomez F & Janeway C A* *Albumin metabolism in children with protein malnutrition.* J clin Invest. *37* 682 1958

58 *Gitli D Kli nbe g J & Hughes W* Site of catabolism f serum albu m n. Nature. *181* 1064 1958

59 *G tlin D Landing, B H & Whipple A* The localisation of homologous plasma proteins *in the tissues of* young human beings as demonstrated with fluorescent antibodies. J exp Med. *97* 163 1953

60. *G tlin, D Nakasato D & Richardso H* Myoalbumin, plasma albumin

and interstitial fluid in human and rabbit muscles J clin. Invest. 34 935 1955
61 Glass G B. & Ishimori, A. Passage of serum albumin into the stomach. Amer J dig. Dis. 6 103. 1961
62 Goldsworthy P D & Volweiler W Comparative metabolic rate of chemically and biosynthetically labelled proteins. Ann N Y Acad. Sci. 70 26. 1957
63 Gordon A H The use of the isolated perfused liver to detect alterations to plasma proteins. Biochem. J 66 255 1957
64 Gordon, A H & Freeman, T Human and rat intestine as a site of catabolism of albumin. Protides of the biological fluids. XI Colloquium. Elsevier Publ. Co Amsterdam. 1963
65 Gordon, R. J The preparation of radioactive polyvinyl pyrrolidone for medical use. J polymer. Sci. 31 191 1958.
66. Gordon R J Exudative enteropathy Lancet 1 325. 1959
67 Gordon R. J Bartter F C & Waldman Th. Idiopathic hypoalbuminaemias Clin. Staff. Conf. N I.H. Ann. intern. Med. 51 553 1959.
68 Grabar P & Williams C A Méthode immunoélectrophorétique d'analyse de mélanges de substances antigéniques. Biochim. biophys. Acta. 17 67 1955
69 Gray H Anatomy of the human body 24th ed Lea & Febiger Philadelphia 1942
70 Gray S J & Frank, H The simultaneous determination of red cell mass and plasma volume in man with radioactive sodium chromate and chromic chloride J clin. Invest 32 1000 1953
71 Gregersen M I A practical method for the determination of blood volume with the dye T 1824 J Lab clin Med 29 1266. 1944
72 Grotte G Passage of dextran molecules across the blood-lymph barrier Acta chir scand. suppl. 211 1956.
73 Gullberg R. & Olhagen, B Electrophoresis of human gastric juice. Nature 184 1848. 1959
74 Hanngren, A Hansson, E., Ullberg, S & Åberg, B. Fat of injected dextran labelled with tritium in mice. Nature. 184 373 1959
75 Haurowitz, F Fleischer S Walter H & Lietze A. The metabolism of isotopically labelled plasma proteins. U N Peaceful use of atomic energy Geneva. 25 111 1958.
76. Heiskell Ch., Wad T Stempien S., Fukuda, M Nakagawa Sh., Yachi, A. & Carpenter Ch. Normal serum proteins in gastric juice. Gastroenterology 40 775 1961
77 Hirsch J Ahrens E. H & Blankenhorn D H Measurement of the human intestinal length in vivo and some causes of variation. Gastroenterology 31 274. 1956.
78. Hoffenberg, R., Saunders, S Linder G C., Black E. & Brock J F I[131]-albumin metabolism in human adults after experimental protein depletion and repletion. p. 314 Protein metabolism. Internat. Symp. 1962 Editor F Gross. Springer Verlag. Berlin. Göttingen. Heidelberg. 1962.
79 Holm I Comparative studies of the plasma proteins on peroral, intravenous, intraportal and enteral administration of casein hydrolysate. Acta chir scand. Suppl. 325 108. 1964
80. Holman, H Nickel W F J & Sleisenger M H Hypoproteinaemia antedating intestinal lesions and possibly due to excessive serum protein loss int the intestine. Amer J Med. 27 963 1959.
81 Huggins R. A., Smith, E. L. & Deavers S Volume distribution of Evans blue dye and iodinated albumin in the dog. Amer J Physiol. 205 351 1963
82 Humphrey J H Ansberger A. & Perkins D J Observations on the

relationship of the rates of serum protein metabolism, heterologous serum protein catabolism and the time and magnitude of the antibody response. Ann. N. Y. Acad. Sci. [illegible] 19[illegible]

41. Dreisbach, L. & [illegible] E. S. Absorption of carbohydrate and protein as affected by feeding cornstarch, banana or glucose. J. Nutr. 53 [illegible] 1954.

42. Elman, R. Parenteral alimentation in surgery. Paul Hoeber Inc., New York, [illegible]

43. Ernster, [illegible], Lindqvist, A., Persson, E. & Wetterfors, J. Tuberous sclerosis with a localized lymphomatous malformation in the jejunum and excessive albumin loss into the lower intestinal tract. Pediatrics 3[illegible] [illegible] 1962.

44. Fine, J. & Seligman, A. M. Traumatic shock. VII. A study of the problem of the "lost plasma" in haemorrhagic, tourniquet and burn shock by the use of radioactive iodo-plasma protein. J. clin. Invest. 23 [illegible] 1944.

45. Fisher, R. B. & Parsons, D. S. The gradient of mucosal surface area in the small intestine of the rat. J. Anat. (Lond.) 84 272, 1950.

46. Franks, J. J., Edwards, K. W., Lathem, W. W. & Freeman, J. B. The role of the gut in albumin catabolism. II. Studies in enterectomized animals. J. gen. Physiol. 4[illegible] [illegible] 1963.

47. Franks, J. J., Mosser, E. L. & [illegible] G. L. The role of the gut in albumin catabolism. I. Studies in the hepatectomized [illegible] rabbits. J. gen. Physiol. 4[illegible] 415, 1963.

48. Freeman, W. L. Lymphatic pathways from the intestine in the dog. Anat. Rec. 82 [illegible] 194[illegible]

49. Freeman, T. & Gordon, A. H. Metabolism of albumin and gammaglobulin in protein deficient rats. Clin. Sci. 2[illegible] 1[illegible] 19[illegible]

50. Freeman, T., Gordon, A. H. & Humphrey, J. H. Distinction between catabolism of native and denatured proteins by the isolated perfused liver after carbon loading. Brit. J. exp. Path. [illegible] [illegible]

51. Freeman, T. & Matthews, C. [illegible] Freeman, A. S., [illegible] H. & Kallee, E. Albumin labelled with iodine [illegible] in an analbuminaemic subject. Nature [illegible] [illegible] 1959.

52. Frenkel, M. & Isselbacher, S. H. Concentration gradients for inorganic I[illegible] and chloride in mixed human saliva. J. clin. Invest. [illegible] [illegible]

53. Friedberg, W. Relation between protein and catabolism of albumin in the mouse. Amer. J. Physiol. 2[illegible] [illegible] [illegible]

54. Friedberg, W., Walker, H. & Hamilton, F. Fate in rats of heterologous protein labelled internally by S[illegible] and externally by I[illegible]. Science [illegible] 1[illegible]

55. Gibson, J. J. & Evans, W. J. Clinical studies of blood volume. Relation of plasma and total blood volume to venous pressure, blood velocity, physical measurements, age and sex in 9[illegible] normal humans. J. clin. Invest. [illegible] [illegible] [illegible]

56. Gitlin, D. Distribution dynamics of circulating and extravascular I[illegible] plasma proteins. Ann. N. Y. Acad. Sci. 1[illegible] [illegible]

Gitlin, D., Cravioto, J., Frenk, S., Montano, E. L., Galvan, R. R., Gomez, F. & Janeway, C. A. Albumin metabolism in children with protein malnutrition. J. clin. Invest. [illegible] [illegible] [illegible]

Gitlin, D., Klinenberg, J. & Hughes, W. Site of catabolism of serum albumin. Nature 181 1[illegible]4 1958.

Gitlin, D., Landing, B. H. & Whipple, A. The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies. J. exp. Med. [illegible] [illegible] 19[illegible]

60. Gitlin, D., Nadas, D. & Richardson, W. [illegible] [illegible] plasma albumin

and interstitial fluid in human and rabbit muscles. J clin. Invest. *34* 935 1955

61 *Glass O B & Ishmori, A.* Passage of serum albumin into the stomach. Amer J dig. Dis. *6* 103 1961

62. *Goldsworthy P D & Volwiller W* Comparative metabolic rat of chemically and biosynthetically labelled proteins. Ann. N Y Acad Sci. *70* 26 1957

63. *Gordon, A. H* The use of the isolated perfused liver to detect alterations to plasma proteins. Biochem. J *66* 255 1957

64. *Gordon A. H & Freeman, T* Human and rat intestine as a site of catabolism of albumin. Protides of the biological fluids. XI Colloquium. Elsevier Publ. Co. Amsterdam 1963

65 *Gordon R. J* The preparation of radioactive polyvinyl pyrrolidone for medical use. J polymer Sci. *31* 191 1958.

66 *Gordon, R. J* Exudative enteropathy Lancet *1* 325. 1959

67 *Gordon R J Bartter F C & Waldmann Th* Idiopathic hypoalbuminaemias. Clin. Staff Conf. N I. H. Ann. intern. Med *51* 553. 1959

68 *Grabar P & Williams, C A.* Méthode immunoelectrophorétique d'analyse de mélanges de substances antigeniques. Biochim. biophys Acta. *17* 67 1955

69 *Gray H* Anatomy of the human body 24th ed Lea & Febiger Philadelphia 1942.

70 *Gray S J & Frank H* The simultaneous determination of red cell mass and plasma volume in man with radioactive sodiumchromat and chromic chloride. J clin. Invest. *32* 1000. 1953

71 *Gregersen, M I* A practical method for the determination of blood volume with the dye T 1824 J Lab. clin Med *29* 1266. 1944

72 *Grotte G* Passage of dextran molecules across the blood-lymph barrier Acta chir scand. suppl. 211. 1956.

73 *Gullberg R. & Olhagen, B.* Electrophoresis of human gastric juice. Nature *184* 1848. 1959

74 *Hanngren A Hansson, E., Ullberg, S & Åberg, B.* Fat of injected dextran labelled with tritium in mice. Nature. *184* 373 1959

75 *Hanrowitz, F Fleischer S Walter H & Leiter A* The metabolism of isotopically labelled plasma proteins. U N. Peaceful use of atomic energy Geneva. *25* 111 1958.

76. *Heiskell, Ch., Wada, T Sternberg, S Fukuda M Nakagawa, Sh., Yachi, A & Carpenter Ch* Normal serum proteins in gastric juice. Gastroenterology *40* 775. 1961

77 *Hirsch, J Ahrens E. H & Blankenhorn, D H* Measurement of the human intestinal length in vivo and some causes of variation. Gastroenterology *31* 274. 1956.

78. *Hoffenberg, R., Saunders S Linder G C Black E. & Brock, J F* I[131]-albumin metabolism in human adults after experimental protein depletion and repletion. p. 314 Protein metabolism. Internat. Symp. 1962. Editor F Gross. Springer Verlag Berlin. Göttingen. Heidelberg. 1962.

79 *Holm I* Comparative studies of the plasma proteins on peroral, intravenous, intraportal and enteral administration of casein hydrolysate. Acta chir scand. Suppl. 325 108. 1964

80. *Holman H Nickel W F J & Sleisenger M H* Hypoproteinaemia antedating intestinal lesions and possibly due to excessive serum protein loss int the intestine. Amer J Med. 27 963 1959

81 *Huggins R. A Smith E. L. & Deavers, S* Volume distribution of Evans blue dye and iodinated albumin in the dog Amer J Physiol. 205 351 1963

82. *Humphrey J H Neuberger A & Perkins D J* Observations on the

relationship of the rates of serum protein metabolism, heterologous serum protein catabolism and the time and magnitude of the antibody response. Ann. N.Y. Acad. Sci. 70 69 1957

41 Dienbach L. & [illegible] E. S. Absorption of carbohydrate and protein as affected by feeding cornstarch, banana or glucose. J. Nutr. 53 523 1954

42. Elman, R. Parenteral alimentation in surgery. Paul Hoeber Inc. New York. 194

43 Enst om [illegible] Lyunggvist A., Persson B. & Wetterfors J. Tuberous sclerosis with a localized anomalous malformation in th ileum and excessive albumin loss into the lower intestinal tract. Pediatrics. 30 971 1962.

44. Fine J. & Seligman A. M. Traumatic shock. VII. A study of the problem of the "lost plasma" in haemorrhagic tourniquet and burn shock by the use of radioactive iodo-plasma protein. J. clin. Invest. 23 720 1944

45 Fisher R. B. & Parsons D. S. The gradient of mucosal surface area in the small intestine of the rat. J. Anat. (Lond.) 84 272, 1950.

46. Franks J. J., Edwards K. W. Lackey W. W. & Fitzgerald J. B. The role of the gut in the albumin catabolism. II. Studies in enterectomized rabbits. J. gen. Physiol. 46 427 1963

47 Franks J. J. M[illegible] E. L. & Anstadt G. L. The role of the gut in albumin catabolism. I. Studies in the jejunoileo-ectomized rabbits. J. gen. Physiol. 46 415 1963

48. Freeman, W. L. Lymphatic pathways from the intestine in the dog. Anat. Rec. 82 543 1942

49 Freeman, T. & Gordon A. H. Metabolism of albumin and gammaglobulin in protein deficient rats. Clin. Sci. 25 17 1964.

50. Freeman, T., Gordon, A. H. & Humphrey J. H. Distinction between catabolism of native and denatured proteins in the isolated perfused liver after carbon loading. Brit. J. exp. Path. 39 4 9 1958.

51 Freeman T. & Matthews C. M., McFarlane A. S., Bernhold H. & Kallee E. Albumin labelled with iodine 131 in an analbuminaemic subject. Nature. 183 606, 1959

52. Frenkel N. & Ingber S. H. Concentration gradients for inorganic I[131] and chloride in mixed human saliva. J. clin. Invest. 32 1077 1953

53 Friedberg, W. Relation between pool size and catabolism of albumin in the mouse. Amer. J. Physiol. 204 501 1963

54 Friedberg W. Walter H. & Haurowitz, F. Fate in rats of heterologous protein labelled internally by S[35] and externally by I[131]. Science. 121 871 1955

55 Gibson, J. Jr. & Evans W. Jr. Clinical studies of blood volume, relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in 90 normal humans. J. clin. Invest. 16 31 193

56 Gitlin D. Distribution dynamics of circulating and extravascular I[131] plasma proteins. An. N. Y. Acad. Sci. 0 122. 195

57 Gitlin D. C[illegible] J. Frenk S. Montaño E. L. Galtan R. R. Gomez, F. & Janeway C. A. Albumin metabolism in children with protein malnutrition. J. clin. Invest. 37 682 1958

58 Gitlin, D. Klinenberg J. & Hughes W. Site of catabolism of serum albumin. Nature 181 1064 1958

59 Gitlin D., Landing B. H. & Whipple A. The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies. J. exp. Med. 97 163 1953

60 Gitlin, D. Nakasato D. & Richardson W. Myoalbumin, plasma albumin

105. *Matthews, C M Ben e-Robertson A & H mphrey J H* Measurement of extravascular albumin in normal rabbits and rats Plasma Proteins and Gastrointestinal tract in health and disease. Proc. Internat. Symp. Paris 1961 Munksgaard. Copenhagen 1962.
106. *Matthews C M* Application of an analogue computer to analysis of ex periments with [131]I-labelled plasma proteins when pools are not in dynamic equilibrium. 1964 In press.
107 *Matthew C M* Personal communication 1964.
108. *Maurer W & Müller E. R.* Albumine als Vorstuf von Globulinen im normalen Organismus der Ratte. J clin. Invest. *34* 1126. 1955.
109 *Mayerson H S Wolfram C G Shirley H J & Wasserman, K.* Regional differences in capillary permeability Amer J Physiol. *198* 155. 1960.
110 *McFarlane A. S* Labelling of plasma proteins with radioactiv iodine. Biochem J 62 135. 1956.
111 *McFarlane A. S* Use of labelled plasma proteins in the study of nutritional problems. Progr. Biophys. 7 116 1957
112 *McFarlane A S* Efficient tracer-labelling of plasma proteins. Nature. *182* 53 1958.
113 *M Farlane A. S* Catabolism of plasma proteins. Lancet. *I* 131 1963
114. *M Farlane A S* Absolute measurements of synthesis ra es of liver-produced plasma proteins Biochem. J 87 221 1963
115 *Miller L L & Bale W F* Synthesis of all plasma protein fractions except gammaglobulins by the liver The use of zone lectrophoresis and lysine a C t define the plasma proteins synthesized by the isolated perfused liver J exp. Med. 99 125. 1954
116 *Nasset E. S* Role of the digestiv ract in the utilization of protein and amino acids. J A. M. A. *164* 172 1957
117 *Nasset E S & Ju, J S* Mixture of endogenous and exogenous protein in the alimentary tract. J Nutr *74* 461 1961
118. *Netsky M G & Leiter S S* Capillary permeability to horse proteins in burn-shock. Amer J Physiol. *140* 1. 1943
119 *Nikles A. & Maurer W* Messung der Neubildungs- und Abbaugeschwindigkeit einzelner Serumeiweissfraktionen nach Gabe on S^{35}-methionine. Verh. dtsch. Ges. inn. Med. *59* 319 1953.
120. *Noble R. P & Gregersen, M I* Blood-volum in clinical shock. I. Mixing time and disappearance rat of T 1824 in normal subjects and in patients in shock, determination of plasma volume in man from 10-minute sample. J clin. Invest. *25* 158. 1946.
121. *N zdor F* Unpublished data.
122. *Nylander G & Olerud S* The vascular pattern of an isolated jejunal loop. Acta chi scand. *121* 39 1961
123 *Orff K. & König, A.* Untersuchungen mit radioaktiven Serumeiweissfraktionen. V Lokale Kapillarpermeabilität und austauschbares Albumin in erschiedenen Organen der Ratte. Z. ges exp Med. *127* 484 1956.
124 *Ouchterlony O* Antigen-antibody reaction in gels. Acta path. microbiol. scand. *32* 231 1953
125 *Palmer R. F & Sullivan M F* Effect of intestinal tract irradiation on serum proteins of the rat. Proc. Soc. exp. Biol. *101* 326. 1959
126. *P ppenheimer J R.* Passage f molecules through capillary walls. Physiol. Rev 33 387 1953
127 *Penn, N Mandeles S & Anker S* On the kinetics of turnover of serum-albumin. Biochim. biophys. Acta. 26 349 1957
128 *Pereira G* The ffect of significant weight change on the predicted plasma olume. J clin. Invest. 25 401 1946.
129 *Perath B* Blood volume determina tions with the Evans blue dye meth-

presence of plasma proteins in skin and tendon. Biochem. J 66 390 1957

83 *Hügli H & v Gunten H R.*, ^{131}I labelled polyvinylpyrrolidone. Plasma proteins and Gastrointestinal Tract in Health and Disease. Proc. Internat Symp Paris 1961 Munksgaard Copenhagen. 1962.

84 *Jaffé R.* Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Julius Springer Verlag Berlin. 1931

85 *Jarnum S.*, Protein-losing gastroenteropathy Blackwell Scientific Publ. Oxford. 1963

86. *Jarnum S & Schwartz, M* Bestemmelse af albumin omsaetningen med ^{131}I-maerket albumin. Metoder till beregning av albuminomsaetningen og nogle klinisk vigtige anvendelsesområ der Nord. Med. 63 708. 1960.

87 *Jeejeebhoy K N* Cause of hypoalbuminaemia in patients with gastrointestinal and cardiac disease. Lancet I 345 1962.

88. *Jeejeebhoy K N & Coghill N F* The measurement of gastrointestinal protein loss by a new method Gut. 2 123 1961

89 *Jensen D & Tarver H* Protein-synthesis in perfused rat liver J gen. Physiol. 39 567 1956.

90. *Job Th* The adult anatomy of the lymphatic system in the common rat Anat. Rec 9 447 1918.

91 *Jones H J & Morgan D B* Measurement of plasma protein loss into gastrointestinal tract using ^{131}I labelled proteins and oral Amberlite resin. Lancet I 626 1963

92. *Katz, J R senfeld Sh. & Sellers A. L.* Role of the kidney in plasma albumin catabolism. Amer J Physiol. 198 814 1960

93. *Katz J Rosenfeld Sh & Sellers A L.* Site of plasma albumin catabolism in the rat. Amer J Physiol. 200 1301 1961

94 *Kimbel K H., Heinkel K. & Börner W* Über die Herkunft des Eiweisses in Magensaft. Untersuchungen mit markierten Humanalbumin. Klin. Wschr 11 602. 1956.

95 *Knisell L. W Margen, S Tarver H Frantz, J Flanagan E K., Hutchin, M E Michaels G D & McCallie D P.*, Studies in methionine metabolism III The fate of intravenously administered S^{35}-labelled methionine in normal adult males, in patient with chronic hepatic disease, »idiopathic" hypoproteinaemia and Cushing syndrome. J clin. Invest. 29 238. 1950

96. *Kleiber M.*, Body size and metabolic rate. Physiol. Rev 27 511 1947

97 *Kobler H & Jeanet F* Bestimmung des Umsatzes von Plasmaalbumin beim Menschen mit einem schweizerischen Präparat von humanen Albumin-I^{131} und Versuch einer Berechnung des intestinalen Anteils am Gesamtumsatz von Albumin. Schweiz. med. Wschr 92 1391 1962.

98. *Lee F C* Changes in the number of small lymphocytes of the blood following ligation of the thoracic duct. J exp. Med. 36 247 1922

99 *Lewallen Ch., Berman M & Rall J* Studies of iodoalbumin. I A mathematical approach to the kinetics. J clin. Invest. 38 66. 1959

100. *Lukin L.* The effects of total-body X irradiation on blood-volume. Radiat. Res. 7 150. 1957

101 *Lukin, L. & Gregersen M I* Praemortal blood-volumes in splenectomized dogs after total-body X-irradiation. Radiat Res. 7 161 1957

102 *Margen, S & Tarver H* Comparative studies on the turnover of serum albumin in normal human subjects J clin. Invest. 35 1161 1956.

103 *Matthews C M* The theory of tracer experiments with ^{131}I labelled plasma proteins. Phys. in Med. Biol. 2 36 1957

104 *Matthews C M* Effects of plasmapheresis on albumin pools in rabbits J clin. Invest. 40 603 1961

30 1228. 1951

153 *Sterling, K.* Serum albumin turnover in Laennec cirrhosis as measured by [131]I-tagged ›bumin. J clin. Invest. 30 1238. 1951

154 *Sullivan, M F* Plasma-protein loss after X-irradiation or nitrogen-mustard administration. Radiat. Res 12 447 1960.

155 *Swann, H G Stegall H F Collings D & Miles V A.* Red cell and ›bumin circulation in the ileum. Amer J Physiol. 201 943 1961

156 *Takeda Y* Metabolism and distribution of autologous and homologous albumin-I[131] in the dog. Amer. J Physiol. 206 1223 1964.

157 *Takeda Y & Reeve E. B* Studies of the metabolism and distribution of albumin with autologous I[131]-albumin in healthy men J Lab. clin. Med. 61 183 1963.

158. *T rner H* The Proteins vol 2 Academic Press New York. 1954

159 *Taylo H L, Mickelsen O & Keys A* The effect of induced malaria, acut starvation and semi-starvation on the electrophoretic diagram of the serum proteins of normal young men J lin Invest 28 273 1949

160. *Terres, G H ghes W L & Wolins W* Whole-body measurements of radioactivity as mean of following in vi the degradation of [131]I-labelled proteins in mice Amer J Physiol. 198 1355 1960.

161 *Thomason, D & Schoefield, R.* The uptake of [131]I-tagged protein by Ehrlich ascites umour ells Exp Cell Res 24 457 1961

162 *Thorbeck G J Sebestyen, M Benacerraf B & Greene H* Influence of reticuloendothelial blockage on turnover ra of homologous plasma proteins in mic Proc Soc exp. Biol. 99 439 1958

163 *T oell L & Aberg, B* The carbohydrate content of the gastric juice after intravenous administration of partly hydrolyzed bacterial dextran t hospital cases. Scand. J lin. Lab. Invest. 4 1 1952

164. *Turner P Sentry G S. & O'D nnell P M* Hypoalbuminaemia due to protein loss from gastric carcinoma. Gut. 4 155. 1963

165 *Ullberg, S Birke G Ewaldsson B Hansson E. Liljedahl S-O Plantin L-O & Wetterfors, J* The role of the gastrointestinal tract in the elimination of albumin. Acta med. scand. 167 421 1960.

166 *Veall N & Vetter H.:* Radioisotope techniques in clinical research and diagnosis. Butterworth & Co. Ltd. London. 1958.

167 *Visek W J Whitney I B Kuhn, U S & Comar C L.* Metabolism of Cr[51] by animals as influenced by chemical state. Proc. Soc. exp. Biol 84 610. 1953

168 *Volwiller W Goldsworthy P D M Martin, M P Wood P A., McKay J R., Freemont-Smith, K. & S hork, D F* Turnover rates of various plasma proteins in normal and cirrhotic humans using biosynthetic methods. J clin. Invest. 33 970. 1954.

169 *Volwiller W Goldsworthy P McMartin M Wood P A., M Kay S R. & Freemont-Smith K.* Biosynthetic determination with radioactive sulphur on turnover rates of various plasma proteins in normal and cirrhotic man. J clin Invest. 34 1126. 1955.

170 *Waldmann, Th..* Gastrointestinal protein loss demonstrated by Cr-labelled albumin. Lancet. 2 121 1961

171 *Waldmann, Th., Trier J & Fallon H* Albumin metabolism in patients with lymphoma. J clin. Invest. 42 171 1963

172 *Waldmann, Th. & Wochner R D* The use of [51]Cr-labelled albumin in the study of protein-losing enteropathy Protides of the biological fluids. XI. Colloquium. Elsevier Publ. Co Amsterdam. 1963

173 *Wasserman, K. & Mayerson, H S.* Exchange of albumin between plasma

od Acta med. scand. suppl. 256. *1951*

130 *Prosser C L., Brown F R. Bishop D W Jahn T L. & Wulff V J.* Comparative animal physiology W B Saunders Co Philadelphia. London. 1950

131 *Reeve E. B & Bailey H R* Mathematical models describing the distribution of ^{131}I-albumin in man. J Lab clin. Med *60* 923 1962.

132. *Reeve E B Pearson J R & Martz, D C* Plasma protein synthesis in the liver: Method for measurement of albumin formation in vivo Science. *139* 94 1962

133 *Reeve E B. & Roberts J E.* The kinetics of the distribution and breakdown of ^{131}I-albumin in the rabbit. J gen. Physiol. *43* 415 1959

134 *Reeve E B & Roberts J E* The catabolism of plasma albumin in the rabbit. J gen. Physiol. *43* 445 1959

135 *Renkin, E. M & Pappenheimer J R.* Wasserdurchlässigkeit und Permeabilität der Kapillarwände. Ergebn. Physiol. *49* 59 1957

136. *Rieger A Liljedahl S-O., Plantin, L-O & Birke G* Restitution of blood volume and blood proteins after graded haemoraghe in dogs. To be published.

137 *Rosenfeld Sh Katz, J & Sellers A L.* Effect of nephrectomy on I^{131}-albumin turnover in th dog. J Lab. clin. Med. *59* 381 1962

138. *Rotschild M A., Schreiber S S Oratz, M & McGee H L.* The effect of cortisone and meticortene on album n I^{131} metabolism Clin. Res 6 27 1958.

139 *Ryser H* The measurement of I^{131} serum albumin uptake by tumour cells in tissue culture Lab. Invest. *12* 1009 1963

140 *Ryser H Aub J C & Caulfield J B* Studies on protein uptake by isolated tumour cells. J Cell. Biol. *15* 437 1962

141 *Schwartz, M & Jarnum S* Gastrointestinal proteinloss in idiopathic (hypercatabolic) hypoproteinaemia. Lancet. *I* 327 1959

142. *Schwartz, M & Thomsen B* Idiopathic hypercatabolic hypoproteinaemia case examined by ^{131}I-labelled albumin. Brit. med. J *I* 14 1957

143 *Silvester C F* On the presence of permanent communication between the lymphatic and the venous system at the level of the renal veins in adult South american monkeys. Amer J Anat. *12* 447 1912

144 *Sjöstrand T.* The total quantity of hemoglobin in man and its relation to age sex, body weight and height. Acta physiol. scand. *18* 324 1949

145 *Sjöstrand T* Relationen zwischen Bau und Funktion des Kreislaufsystems und ihre Veränderungen unter pathologischen Bedingungen. Forum Cardiol. Heft 3 Boehringer & Soehne. Mannheim-Waldhof 1961

146. *Sjöstrand T* Personal communication. 1961

147 *Spector W S* Handbook of biological data. W B. Saunders Co. Philadelphia. London. 1956.

148 *Starling, E. H* The fluids of the body Archibald Constable & Co. Ltd. London 1909

149 *Sternfeld J L.* Differences in daily albumin synthesis between normal men and women as measured with ^{131}I labelled albumin. J Lab. clin. Med. *55* 904 1960.

150 *Sternfeld J L Davidson J D & Gordon R Jr* A mechanism for hypoalbuminaemia in patients with ulcerative colitis and regional enteritis. J clin Invest. 36 931 1957

151 *Steinfeld J L., Davidson J D Gordon R J & Greene F E.* The mechanism of hypoproteinaemia in patients with regional enteritis and ulcerative col tis. Amer J Med *29* 405 1960

152 *Sterling K.* The turnover rate of serum albumin in man as measured by ^{131}I tagged albumin. J clin Invest.

and lymph. Amer J Physiol 165 15 1951

174 *Welch C S Adams M & Wakefield E G* Metabolic studies on chronic ulcerative colitis. J clin. Invest 16 161 1937

175 *Wetterfors J & Liljedahl S-O* Hypoproteinemia vid ileus och peritonit. Nord. Med. 68 1669 1962

176 *Yoffey J M & Courtice F C.* Lymphatics, Lymph and Lymphoid tissue. Harvard Univ Press. Cambridge Mass. 1956.

177 *Yuile C L., O Dea A E., Lucas F V & Whipple G H.* Plasma protein labelled with lysine ... Its oral ... ism in the dog J exp. Med. 96 247 1952.

178. *Yuile C L. Lucas F V., Neubecker R D & Whipple G H.* Reduction of extravascular — extracellular proteins in protein-depleted dogs. Fed. Proc. 14 424 1955

179 *Zizza F Campbell T J & Reeve E B.* The nature and rates of excretion of radioactive breakdown products of I^{131}-albumin in the rabbit. J gen. Physiol. 43 397 1959

180 *Åstrand P O.* Experimental studies of physical working capacity in relation to sex and age. Munksgaard, Copenhagen 1952.

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Clinical and Immunoelectrophoretic Studies on Cerebrospinal Fluid in Virus Meningoencephalitis and Bacterial Meningitis

By Bo Ursing

Acta Medica Scandinavica
Supplementum 429

From the Department of Infectious Diseases (Head K. E. Thulin, MD)
and the Department of Neurology (Head. R. Müller Professor)
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INTRODUCTION

Investigation of the cerebrospinal fluid (C.S.F.) proteins is of considerable clinical interest in diseases of the central nervous system (C.N.S.) Dispite recent advances in our knowledge of the C.S.F., it is still not known with certainty how the C.S.F. proteins are formed. According to the present concept, they pass by diffusion into the fluid from the plasma and, in addition to entering by diffusion, certain substances are actively secreted into the C.S.F. by the choroid plexus.

Refined methods useful for the analysis of the C.S.F. are now available. One of these methods is immunoelectrophoresis, which has proved valuable in qualitative studies of the C.S.F. Investigations with this technique have shown, among other things, that the protein pattern of the C.S.F. differs from that of the plasma. The main difference is that the large molecular plasma proteins are normally missing or occur in only insignificant concentration in the C.S.F. and that some proteins differ in appearance and position from those in plasma, *e.g.*, prealbumin, α_1-glykoprotein, transferrin, and γ_{2}-globulin.

In infections of the C.N.S. the blood-C.S.F. barrier is disturbed, and it is now known that the immunoelectrophoretic protein pattern of the C.S.F. changes and tends to resemble that of the plasma. But no systematic attempts have been made to find out which plasma proteins pass into the C.S.F., when this transfer occurs, or how long it lasts.

In certain chronic diseases involving the C.N.S. e.g. multiple sclerosis and subacute sclerosing leucoencephalitis the γ-globulin content of the C.S.F. is increased without any co-existing increase of the component in the plasma. It is however not known to what extent the γ-globulin content of the C.S.F. is increased in the acute, benign types of meningoencephalitis, which are caused chiefly by enterovirus and mumps virus, and which are the most common infections of the C.N.S. in Europe. The C.S.F. has been systematically studied previously with paper electrophoresis, which, however shows only relatively large changes in the concentration of the various components. Immunoelectrophoresis, on the

HISTORICAL

Immunoelectrophoresis was introduced by GRABAR and WILLIAMS (1953) who combined electrophoretic separation in agar gel (GORDON et al. 1949) with double diffusion (OUCHTERLONY 1948) in one and the same medium. With this method it is possible to analyse a mixture of proteins by utilizing their electrophoretic and antigenic properties. Microimmunoelectrophoresis, a modification described by SCHEIDEGGER (1955) has great advantages it is time-saving and requires only 0.5 % of the amount of antigen and 1 % of the amount of antiserum necessary for the conventional method. With this technique it has been possible to separate as many as 25 distinct antigenic proteins in the serum (HEREMANS 1963) where each electrophoretic region shows multiple antigen-antibody precipitates.

The first examinations of the C.S.F were published by GAVRILESCO et al. (1955) 2 years after introduction of immunoelectrophoresis. They studied persons with diseases known not to involve the C.S.F Parallel analyses using horse antiserum were made of the serum and C.S.F The serum pattern showed 16—18 precipitates and the C.S.F pattern about 10. The precipitates of the C.S.F were 1 prealbumin, 2—3 α_1-globulin, 3 α_2-globulin, 1 β_1-globulin, 1 β_2-globulin which consisted of 2 immunologically identical lines and not yet identified as transferrin and a γ-globulin line weaker and shorter than that seen in the serum pattern.

SCHEIFFARTH et al. (1958) reported the first analysis of C.S F from patients with neurological disease, mainly cerebral tumour and compared the results with those obtained in healthy persons. The normally occurring prealbumin was missing in the spectra of the C.S.F of patients with such diseases. Apart from this no qualitative differences were found. It was also noted that normally occurring precipitates were more marked, i.e., a sign of increased concentration of the protein in question. They used antiserum that they had prepared themselves.

The first more differentiated investigations were published by FRICK (1959)

other hand, is capable of demonstrating even very small changes (micrograms) and was therefore considered worth while trying in a more refined investigation of the formation of γ-globulin in the C.N.S

The purposes of the present investigation were the following

1 To study the immunoelectrophoretic protein pattern of the C.S.F for changes in the course of a number of viral and bacterial infections of the C.N.S Interest was focused chiefly on the β_1-lipoprotein, fibrinogen, and γ_1-macroglobulin, not normally occurring in the C.S.F and on the rapidly migrating $\gamma_{\text{[illegible]}}$-globulin, which forms the anodic part of the band containing a larger amount of neuroamino acids and situated in the α_2- and β_1-regions, and on the α_2-macroglobulin which can be demonstrated in some C.S.F samples in only very low concentration.

2 To study these findings for any correlation with other clinical findings.

3 To assess, on the basis of the results obtained under points 1 and 2 the value of immunoelectrophoresis of the C.S.F in the investigation in infections of the C.N.S

DENCKER and SWAHN reported an analysis of more than 400 persons with neurological disorders including epilepsy multiple sclerosis, cerebral tumour polyneuritis and infections of the C.N.S as well as of a control group. As a rule, the controls showed 13—14 precipitates, sometimes occasionally as many as 17 β_1-lipoprotein, γ_1-macroglobulin, and fibrinogen could not be demonstrated. α_2-macroglobulin was seen as a weak precipitate in some, and the anodic part of the γ_{ss}-globulin line was missing. Patients with polyradiculitis (DENCKER et al 1964) tumour of the C.N.S. (SVEDMILSSON et al. 1961) or bacterial meningitis (URSINO et al. 1962) showed the most pathological protein patterns, which in some cases resembled that of the serum. The most remarkable finding in meningoencephalitis was that the γ_1-macroglobulin was often found in the C.S.F and that this change persisted for several weeks after onset of the disease without any co-existing sign of increased concentration of the γ_1-macroglobulin in the serum (URSINO et al. 1962)

The same year LATERRE et al. (1962) reported 1 case of benign lymphocytic meningitis with an isolated increase of the γ_1-macroglobulin in the C.S.F without any accompanying macroglobulinaemia.

It is usually believed that antigens which have the same electrophoretic mobility in the C.S.F and serum also have identical immunological properties. This assumption was colaborated by FRICK and SCHEID-SEYDEL (1957) for albumin, α_1-glycoprotein, transferrin and γ-globulin on the basis of OUCHTERLONY's (1953) criteria for immunological identity By combining the gel diffusion technique and immunoelectrophoresis, so-called combined diffusion method, CLAUSEN (1960) established the identity also of α_1-lipoprotein, α_1-mucoprotein, ceruloplasmin, haptoglobin, α_2-macroglobulin, β_1-lipoprotein, β_1-A-C and γ_1-A-globulin from C.S F and serum. The aforementioned assumption of the immunological identity probably also holds for the other proteins when antihuman serum is used (MACPHERSON and COSGROVE 1961)

and BURTIN (1959 1960) Both used horse antiserum. FRICK found good agreement with previous investigations of normal C.S.F In multiple sclerosis and syphilis, however he observed an increase of the γ-globulin concentration, which was seen as a marked precipitate along its whole length. In some cases a further γ-globulin precipitate was seen, which proved identical with the rapidly migrating anodic part of the γ-globulin line. On examination of a few cases of purulent meningitis he found an increase of the number of α- and β-globulin precipitates. The characteristic β-globulin precipitate—which had by then not been identified as transferrin—could not be demonstrated in patients in whom amounts of serum had been observed to pass into the C.S.F space. The γ-globulin precipitate was also marked in purulent meningitis.

As before, BURTIN found the C.S F in normals to contain 1 prealbumin fraction, albumin, α_1-muco- and α_1-glycoprotein and 2 or 3 precipitates in the α_2-area. α_2-macroglobulin, ceruloplasmin and haptoglobin could, however not be demonstrated. Two β_1-globulins were found, one of which consisted of a double line identified as transferrin. The γ-globulin line was short and consisted of only the cathodic part. The immunoelectrophoretic pattern was normal in patients with normal or only slightly elevated total protein concentration. Those with an increased total protein content of the C.S.F were divided into 2 groups. One of them showed a quantitative increase of the proteins in the normal pattern. The pattern was judged quantitatively from the appearance and position of the precipitates. The second group included patients with quantitative as well as qualitative changes of the protein pattern. Nearly all the serum proteins, with the exception of α_2-macro- and γ_1-macroglobulin and β_1-lipoprotein, were demonstrated. The increase of the γ-globulin concentration was reflected in an increased density and extent of the precipitate. γ-macroglobulin was also demonstrated but in 1 person with macroglobulinaemia.

Our knowledge was widened further mainly by CLAUSEN (1960 1960 a, 1962) CLAUSEN et al. (1962) DENCKER et al. (1960 1961) and DENCKER and SWAHN (1961 1961 a) In 1960 CLAUSEN who worked with horse and rabbit antiserum supplemented with monospecific antiserum reported 16 precipitates in C.S.F concentrated 1000 times. In addition to previously known fractions, haptoglobin, ceruloplasmin, α_2-macroglobulin, β_1-A-C and γ_1-A-globulin were identified. In pathological conditions with "break down" of the blood-C.S.F barrier he found an increase of the α_2-macro precipitate and β_1-lipoprotein in C.S.F concentrated 100—200 times. In association with simultaneous immunisation the γ A and γ_{ss}-globulin precipitates were more marked and the occurrence of γ_1-macroglobulin was noted (1962)

Microimmunoelectrophoresis introduced by SCHEIDEGGER (1955) and modified by HEREMANS (1960) was used. A glass microscopic slide was covered with 2 ml. of 2 % Remagar (Behringwerke, Marburg an der Lahn, Germany) to form a layer about 1 mm. thick. Two holes, about 7 mm. apart, were stamped in the agar gel with a needle. By means of moistened filter paper the connection was established between agar and buffer. The analysis was carried out in a barbiturate buffer of pH 8.2 and ionic strength of 0.05. After electrophoresis for 5 hours at 70 volt a trough parallel to the electric field was charged with 30—35 microlitres of antiserum. The slide was allowed to develop precipitin reactions in humid atmosphere for 2 days. Excess protein from the gel was then eluted by washing the slide in a saline solution for 2—3 days. The preparation was afterwards dried in a thermostat at 37° C.

COLOURING METHODS

In order to demonstrate certain components the following selective stains were used. The proteins were coloured with amido black 10 B for 10 minutes, followed by decolorization with a mixture of water, acetic acid and aetanol for 30 minutes. The lipoproteins were stained with Sudan black for 4 hours and then decolored with 50 % aethanol for 15 minutes.

ANTISERA

Two types of antibodies can be distinguished according to the type of precipitates they form. The R-type (R=rabbit) whose precipitate is practically insoluble in antibody in excess and poorly soluble in antigen in excess, and H-type (H=horse) whose precipitate is readily soluble in both antigen and antibody excess. In double diffusion analysis of complex mixtures of antigen, in which some constituents may be excessive and others occur only in minute amounts, R-type antibodies usually exceed the H-type.

In order to prevent misinterpretation of the results owing to absence or low concentration of antibodies in an antiserum against an antigen in the solution examined, it is advisable to use two different antisera. For this reason as well as to secure uniform evaluation, all samples were analysed not only against commercial rabbit antisera supplied by Behringwerke, Marburg an der Lahn, Germany during the years 1960—1963, but also against antiserum prepared at the laboratory. It was produced by immunising 5 rabbits with human serum and Freund's adjuvans. Each animal was given 30 mg. of protein each time in re

METHOD

Cerebrospinal fluid was obtained throughout by lumbar puncture. As a rule 10—15 ml. was tapped and 5—10 ml. was concentrated No blood-stained samples were accepted Only in 5 cases of virus meningoencephalitis did the number of red cells exceed the suggested limit of 500 per cu.mm. (CLAUSEN et al. 1964) The largest number seen in these 5 cases was 850 cells per cu.mm. In bacterial meningitis, where puncture haemorrhage is more common, a larger number of red cells was tolerated. Nine persons had between 500 and 1500 red cells per cu.mm.

In order to eliminate any admixture of cell proteins the C.S F was centrifuged immediately after collection. The concentration was done according to MIES (1953) in a collodium bag (*Membranfiltergesellschaft, Göttingen, Germany*) Concentration was continued for 2—4 hours until 60—70 microlitres of 0 5—4 % concentrate remained. Five microlitres of concentrate was diluted to 5 ml. after which its extinction was measured in a Beckman DU in the UV region, according to WADELL (1956)

The amount of C.S.F protein concentrate used for immunoelectrophoresis was intended to correspond to the amount of a serum sample with 7 g % protein, corresponding to about 50 micrograms. In order to obtain the desired amount of protein for analysis it was necessary repeatedly to replenish the hole, which held about 0 65 microlitres. This is possible because the solution is rapidly absorbed by the gel (HEREMANS 1960) When the hole had to be filled more than 3 times, a hole about 3 times as large *i.e.* about 2 microlitres, was used. Undiluted serum and the corresponding amount of serum protein in a diluted solution and applied by repeated filling of the hole gave identical immunoelectrophoretic patterns. (Fig 2) Series where substantially larger or smaller amounts of antigen were used did not show any noteworthy change in the appearance or number of the precipitation lines. This is shown in Fig 3 where 60 mg., 50 mg 40 mg and 30 mg respectively were analysed.

over it has been shown (GRABAR 1958) that all macromolecules diffuse slowly which explains why α_2-macroglobulin, β_1-lipoprotein and γ_1-macroglobulin are seen close to the diffusion centre of the antigen.

Appearance of precipitates The precipitate can usually be described as symmetric or asymmetric, markedly or slightly arcuate and as long or short (HIRSCHFELD 1960). A symmetric precipitate is defined as a precipitate where the point nearest the antibody trough coincides with the centre of the precipitate. The curvature of a precipitate depends on the relation between the rate of diffusion of the antigen and of the antibodies, which in turn depends upon the concentration and molecular weight. If the antigen diffuses much slower than the antibodies the curvature of the precipitate will be marked, *e.g.* β_1-lipoprotein (KORNGOLD and LEEUWEN 1957). A long, only slightly arcuate band *e.g.* γ_{ss}-globulin suggests that the precipitate is formed by several antigens which are electrophoretically heterogeneous but immunologically identical (WILLIAMS and GRABAR 1955).

In this investigation interest was focused on 5 proteins α_2-macroglobulin, β_1-lipoprotein, fibrinogen, γ_1-macroglobulin, and γ_{ss}-globulin.

α_2-MACROGLOBULIN

α_2-macroglobulin has a molecular weight of about 900,000. The large molecular 19 S fraction prepared by ultracentrifugation consists to 65 % of α_2-macroglobulin and to 35 % of γ_1-macroglobulin. The concentration of this protein in the serum, which is the same in newborns as in adults, corresponds to 80 % of the entire α_2-fraction.

This protein is easy to identify in the serum. It has an electrophoretic mobility placing it in the α_2-group, which in immunoelectrophoresis corresponds to the area somewhat in front of the antigen hole. Owing to its molecular size the protein diffuses slowly and the precipitate therefore lies far from the antiserum trough even when the concentration of the α_2-macroglobulin is high. Its curvature is marked owing to its high molecular weight in relation to that of the antibodies. In the C.S.F., where the concentration is normally very low the shape of the precipitate is by no means so characteristic as in serum. As mentioned previously a purified commercial antiserum from Behringwerke was therefore used. Weak precipitates, which may occur in normal C.S.F. were ignored, while precipitates reminiscent of those seen in serum, namely marked α_2-macroglobulin precipitates, were classified as positive findings (Fig. 4).

peated subcutaneous injections after which a pool was prepared from the 4 best bleedings.

Monospecific sera against a protein can be *prepared and used in the analysis* of protein mixtures. The simple band formed this way should correspond to one of the bands formed when complete antiserum is used. Purified commercial monospecific antiserum from Behringwerke was used to check the *identity of* α_2-macroglobulin and fibrinogen. All samples were thus analysed with anti α_2-macroglobulin serum No. 329 A. The specimens which showed no precipitate on first analysis i.e. routine analysis during the years 1960—1963 with anti-fibrinogen serum were re-analysed once with a new antifibrinogen serum No. 333 B

In the course of the investigation duplicate determinations were often made to check the reliability of the method. The double determinations were identical throughout.

In parallel with the analysis of the C.S.F., the serum was, as a rule, studied immunoelectrophoretically with commercial antiserum as well as with antiserum prepared at the laboratory

ANALYSIS OF IMMUNOELECTROPHORETIC PATTERN

In the interpretation of the immunoelectrophoretic pattern *i.e.* identification of the substances responsible for the bands, the following criteria were used.

Electrophoretic position. Several precipitates can be identified from their electrophoretic position alone. *According to* GRABAR (1964) there is, as a rule, a close correlation between the relative rate of migration of a protein in agar gel and in liquid medium. This rule is, however, not without exceptions, one of which is undenaturated fibrinogen, which migrates relatively slowly in agar gel (WIEME 1957)

Position of precipitates between diffusion centres. The position of a precipitate relative to its diffusion centre depends on certain factors. Thus, the rate of *diffusion is* dependent on the concentration of agar The higher the concentration the slower the molecules diffuse through the gel. The position of a precipitate also varies with the concentration of *the reactants, the rate of* diffusion increasing with the concentration of the agent (OUCHTERLONY 1958) More-

over it has been shown (GRABAR 1958) that all macromolecules diffuse slowly which explains why α_2-macroglobulin, β_1-lipoprotein and γ_1-macroglobulin are seen close to the diffusion centre of the antigen.

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α_2-MACROGLOBULIN

α_2-macroglobulin has a molecular weight of about 900,000. The large molecular 19 S fraction prepared by ultracentrifugation consists to 65 % of α_2-macroglobulin and to 35 % of γ_1-macroglobulin. The concentration of this protein in the serum, which is the same in newborns as in adults, corresponds to 80 % of the entire α_2-fraction.

This protein is easy to identify in the serum. It has an electrophoretic mobility placing it in the α_2-group, which in immunoelectrophoresis corresponds to the area somewhat in front of the antigen hole. Owing to its molecular size the protein diffuses slowly and the precipitate therefore lies far from the antiserum trough even when the concentration of the α_2-macroglobulin is high. Its curvature is marked owing to its high molecular weight in relation to that of the antibodies. In the C.S.F., where the concentration is normally very low, the shape of the precipitate is by no means so characteristic as in serum. As mentioned previously, a purified commercial antiserum from Behringwerke was therefore used. Weak precipitates, which may occur in normal C.S.F. were ignored, while precipitates reminiscent of those seen in serum, namely marked α_2-macroglobulin precipitates, were classified as positive findings (Fig. 4).

peated subcutaneous injections after which a pool was prepared from the 4 best bleedings.

Monospecific sera against a protein can be prepared and used in the analysis of protein mixtures. The simple band formed this way should correspond to one of the bands formed when complete antiserum is used. Purified commercial monospecific antiserum from Behringwerke was used to check the identity of α_2-macroglobulin and fibrinogen. All samples were thus analysed with anti α_2-macroglobulin serum No. 329 A. The specimens which showed no precipitate on first analysis i.e. routine analysis during the years 1960—1963 with anti-fibrinogen serum were re-analysed once with a new antifibrinogen serum No. 333 B

In the course of the investigation duplicate determinations were often made to check the reliability of the method. The double determinations were identical throughout.

In parallel with the analysis of the C.S.F., the serum was, as a rule, studied immunoelectrophoretically with commercial antiserum as well as with antiserum prepared at the laboratory

ANALYSIS OF IMMUNOELECTROPHORETIC PATTERN

In the interpretation of the immunoelectrophoretic pattern *i.e* identification of the substances responsible for the bands, the following criteria were used.

Electrophoretic position. Several precipitates can be identified from their electrophoretic position alone. According to GRABAR (1964) there is, as a rule, a close correlation between the relative rate of migration of a protein in agar gel and in liquid medium. This rule is, however not without exceptions, one of which is undenaturated fibrinogen, which migrates relatively slowly in agar gel (WIEME 1957)

Position of precipitates between diffusion centres The position of a precipitate relative to its diffusion centre depends on certain factors. Thus, the rate of diffusion is dependent on the concentration of agar The higher the concentration the slower the molecules diffuse through the gel. The position of a precipitate also varies with the concentration of the reactants, the rate of diffusion increasing with the concentration of the agent (OUCHTERLONY 1958) More-

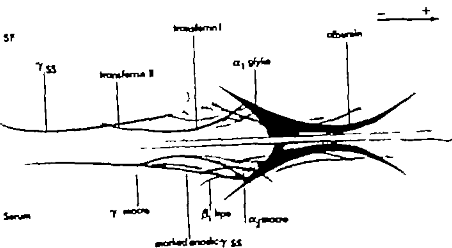


Fig. Normal immunoelectrophoretic pattern of C.S.F. and serum coloured with amido black. Rabbit-antihuman-antiserum.

β_1-LIPOPROTEIN

β_1-lipoprotein, which has a molecular weight of at least 1 300 000 consists to 75 % of lipoids. In immunodiffusion of β_1-lipoprotein a few special points should be borne in mind. Firstly this molecule diffuses slowly, like all macromolecules, and secondly β_1-lipoprotein precipitates locally through the sulphate groups of the agar gel (WIEME 1959) This chemical precipitation has no demonstrable effect on the specific immunoreaction (HITZIG 1961) Moreover its apparent mobility is also dependent on its concentration the higher the concentration the lower its mobility (URIEL and GRABAR 1956) The precipitate is located near the antigen hole, but far from the antibody trough (Fig 5) It stains readily with Sudan black but if it is present in only low concentration, it is not well defined.

The difference in rate of migration of this protein in two media has resulted in a certain confusion of the nomenclature, and in the literature such names are found as α_2-lipoprotein (HIRSCHFELD 1960 CLAUSEN 1960 a, CROWLE 1961) and β_1 lipoprotein (DENCKER and SWAHN 1961 ROSENTHAL and SOOTHILL 1962) and lipoprotein lente (BURTIN 1960)

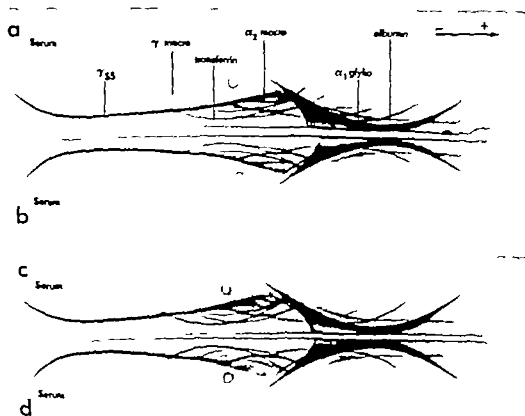
FIBRINOGEN

The fibrinogen molecule is characterised by its distended narrow appearance at about 475 Å. Its molecular weight is about 400 000 In native form the fibrinogen migrates poorly in agar gel which, however is not due to precipitation in the gel (WIEME 1957) As a rule, the precipitate is short and situated between the antigen hole and the trough with the antiserum. Immunologically fibrin does not differ from fibrinogen. Degradation products of fibrinogen also react with antifibrinogen serum, but the precipitate has a different appearance and often also position (SCHULTZE and SCHWICK 1957) After fibrinogenolysis several precipitates can be demonstrated with specific rabbit antisera indicating the presence of different antigen determinants (NUSSENZWEIG and SELIGMANN 1960) All precipitates obtained with antifibrinogen were recorded as positive findings and called fibrinogen precipitates irrespective of the appearance and position of the precipitates (Fig 6)

γ_1-MACROGLOBULIN

This protein, also known as β_2-macroglobulin together with γ_1-A and 7 S γ- or γ_{ss}-globulin (ss = sensu stricto) is now generally called immunoglobulins. HEREMANS (1960) has shown that they have specific determinants as well as de

Fig. 3. Only greater differences in the amounts of antigen used gave noteworthy changes in the immuno-electrophoretic pattern a. 60 mg. b. 5 mg c. 4 mg. d 30 mg



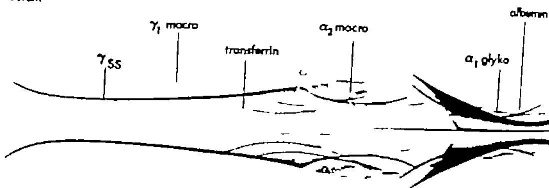


Fig a. Undil ted serum and b. the corresponding amount of serumprotein in a dil ted sol tion ga identical patterns.

β_1 lipoprotein

− +

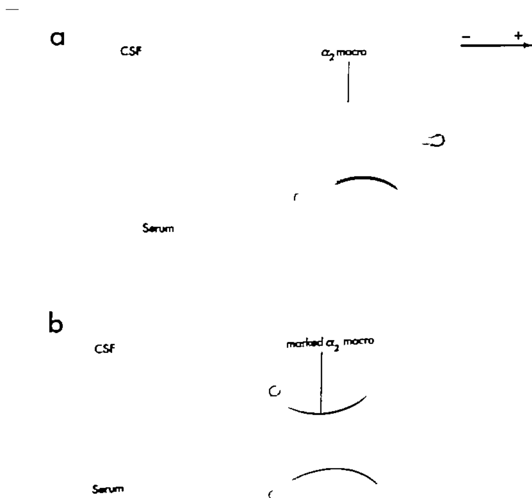
CSF

Serum

β_1 lipoprotein

Fig 5. β -lipoprotein precipitate from case with bacterial meningitis coloured with Sudan black. Rabbit-antihuman-antiserum.

Fig 4. a. Normal α_2-macroglobulin precipitate.
b. Marked α_2-macroglobulin precipitate from a case with bacterial meningitis. Anti-α_2 macroglobulin serum was used



β_1 lipoprotein

– +

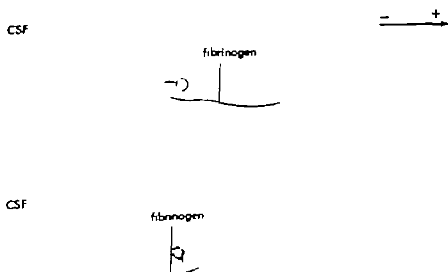
CSF

Serum

β_1 lipoprotein

Fig. 5. β-lipoprotein precipitate from case with bacterial meningitis coloured with Sudan black. Rabbit-antihuman-antiserum.

Fig 6 Fibrinogen precipitates from 2 cases of bacterial meningitis. Antifibrinogen serum was used.



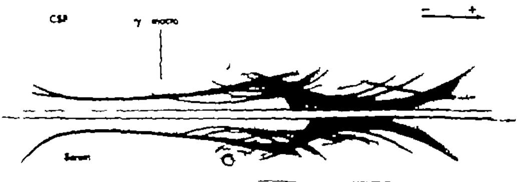
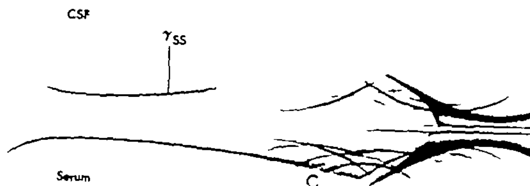


Fig. 7 γ-macroglobulin precipitate from case with virus meningoencephalitis. Rabbit-antihuman-antiserum



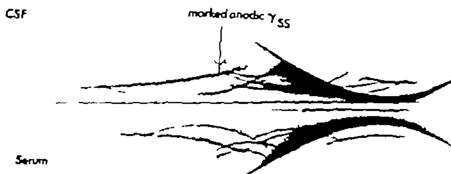


Fig. 8 a. Normal γ_{ss}-globulin precipitate. b. Marked anodic γ_{ss}-globulin precipitate from case with virus meningoencephalitis. Rabbit-antihuman-antiserum.

terminants in common. Recent research has begun to elucidate the chemical structure of these globulins. Reduction and fractionation of γ_1-A- and γ_1-macroglobulins have yielded the same A- and B- chains as γ_{ss}-globulin had formerly been found to consist of. It is held probable that the B-chains are identical in all three types, but that the A-chain differs from one type of immunoglobulin to the other. The antibody locus is either in the B-chain or A-chain or is formed jointly by association of the two chains (cit. PORTER 1963).

γ_1-macroglobulin, which has a molecular weight of about 900,000 is precipitated as a double band in immunoelectrophoresis. The anterior band is arcuate and encloses the antigen hole. The posterior band is only slightly arcuate, except its tail, and extends across the β- and γ-regions. The entire precipitate lies far from the antibody trough, for it is a large molecular protein. Even when the concentration of the γ_1-macroglobulin is high, the precipitate lies close to the antigen hole and it can be readily identified from its appearance and site. Sera from newborns and children show a weak precipitate, or it may be missing as a sign of immunological immaturity (TORLER 1958). It may sometimes be difficult to demonstrate this precipitate also in sera from adults but it will usually appear if larger amounts of serum are used. As mentioned in the introduction, C.S.F. does not normally contain this protein. Among the cases regarded as positive, some showed only one of the γ_1-macroglobulin bands. In most cases, however γ_1-macroglobulin was more or less marked along its entire length (Fig. 7).

γ_{ss}-GLOBULIN

γ_{ss}-globulin or 7 S γ-globulin corresponds to what is usually called γ-globulin. It has a molecular weight of about 150,000 and a sediment constant of 6.8—7 S. The γ_{ss}-globulin precipitate in the serum extends from the cathode into the α_2-area and the anodic part has a higher concentration of neuroamino acids (SCHULTZE 1958). In the serum the precipitate is usually equally marked, but in some cases e.g. in newborns and immunologically immature children that part of the γ-globulin precipitate corresponding to α_2- and β-areas is missing (TORLER 1958). As mentioned in the introduction, this anodal part of the γ_{ss}-globulin precipitate in the normal C.S.F. is also missing. The cases regarded as positive, i.e. those with marked anodic γ_{ss}-globulin precipitate, showed a band that was almost equally marked in the anodal part and in the rest of the precipitate and reached the antigen hole. When the concentration of this antigen was high, the precipitate was seen far from the antigen hole, in other cases closer (Fig. 8.)

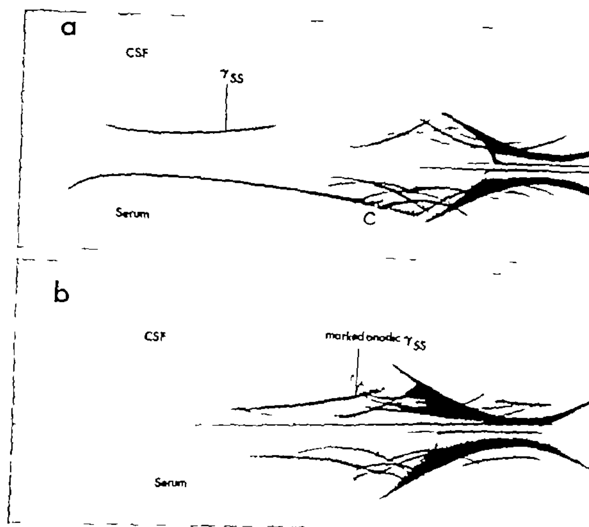


Fig. 8. a. Normal γ_{ss}-globulin precipitate. b. Marked anodic γ_{ss}-globulin precipitate from a case with virus meningoencephalitis. Rabbit-antihuman-antiserum.

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LABORATORY TESTS

The sample was examined immediately after the puncture for the number of cells in a Fuchs-Rosenthal counting chamber

The total protein content was determined by direct measurement in the UV region according to Wadell (1956)

Paperelectrophoresis on serum and C.S.F was performed according to the method described by Laurell, Laurell and Skoog (1956)

CONTROLS

In the present investigation 2 control series were used.

Control series I consisted of 21 patients, admitted to the department of orthopaedics, Lund, because of traumatic injury of the lower limbs. At the time of the examination these patients were healthy apart from the local injury. They varied in age between 18 and 52 years. C.S.F samples were obtained in association with preoperative spinal anaesthesia. Routine analysis of the C.S.F regarding sugar and chloride concentration as well as the cell count showed nothing remarkable.

On statistical treatment of the total protein concentration the mean was found to be 41.9 and the variation 29.9—53.9 mg per 100 ml. (± 2 standard deviation units). The results of the statistical calculation of the sizes of the fractions on paper electrophoresis are given below.

These values agree very well with those from another investigation at this laboratory of 66 persons who sought advice for mild symptoms and who were found to have no neurological disorders (DENCKER 1963). They are also in good agreement with the values in the literature (SCHÖNENBERG 1960).

Immunoelectrophoresis of the C.S.F concerning the five plasma proteins studied gave the following results. Five subjects showed a very weak diffuse precipitate of α_2-macroglobulin, which could only be demonstrated with specific anti α_2-macroglobulin serum. The remaining 16 did not show this precipitate.

Table. Total protein content and paper electrophoresis of C.S.F in 21 control patients

N	Total protein in mg per 100 ml.	Prealbumin per cent	Albumin per cent	α_1 per cent	α_2 per cent	β per cent	β_2 per cent	γ per cent
Mean	4 9	4	63 8	5.6	5	8.0	5.2	0.0
Stand. dev. units	6	0.4	7	.8	.4	2.2	2	0.4

Conventional statistical methods were used (Hill, A. B. Principles of medical statistics, Lancet, London 1956.)

The β_1-lipoprotein could not be demonstrated in the C.S F of any of the subjects. A very weak precipitate with antifribrinogen serum was demonstrated in a 24 year old person. In the remaining 20 subjects no fibrinogen was demonstrable. γ_1-macroglobulin and marked anodic γ_m-globulin precipitates were not found in any of the samples of the C S F

Control series II consisted of 15 patients of the department of infectious diseases in Lund who had been admitted for infections such as bronchopneumonia (5) virosis (4) sinuitis (1) measles (1) otitis (1) orchitis (1) herpes angina (1) mononucleosis infectiosa (1) without neurological signs. The patients' ages ranged from 7 to 53 years. Four had not filled 15 years. None of the patients had more than 5 cells per cu.mm. in the C.S F The concentration of sugar and chloride was normal. Twelve persons had a total protein content of less than 50 mg per 100 ml. In 3 it was 51 53 and 58 mg per 100 ml. The lowest value noted was 28 mg per 100 ml. Paper electrophoresis of the C.S.F showed increased γ-globulin concentration in 1 patient.

On immunoelectrophoresis of the C.S.F 6 persons showed a weak α_2-macroglobulin precipitate. In the remaining 9 this precipitate was missing One patient who had mononucleosis infectiosa had a very weak fibrinogen precipitate in the C.S F No such precipitate was found in any of the remaining 14 subjects. Neither γ_1-macroglobulin nor marked anodic γ_m-globulin precipitate was found in any cases in the C.S.F Sudan staining showed no β_1-lipoprotein in any of the patients.

It is clear from this account of the 36 subjects that the results obtained were in good agreement with those in previous investigations. The immunoelectrophoretic protein pattern of the C S F was characteristic. It was characterised above all by the absence of large plasma proteins with an upper limit of the molecular weight of about 150 000—200 000 That the α_2-macroglobulin could be demonstrated in low concentration was beyond doubt. In 2 cases fibrinogen was noted as a weak precipitate, a finding never reported before. In the patient with infectious mononucleosis a certain meningeal irritation may have been present. In the other case there might have been latent spinal injury Fibrinogen in the C.S.F has been demonstrated in patients with rupture of the intravertebral disk (DENCKER and SWAHN 1961) Neither has β_1-lipoprotein been demonstrated previously by immunoelectrophoresis. The γ_1-macroglobulin in normal C.S.F has been demonstrated only in cases of macroglobulinaemia and myeloma. Neither has marked anodic γ_m-globulin precipitate been found previously in the C.S.F from persons without neurological disorders.

VIRUS MENINGOENCEPHALITIS

Examination at Onset of Disease

MATERIAL

The material consisted of patients admitted during the years 1960 to 1963 to the department for infectious diseases, University Hospital, Lund, to the Children's Hospital, Lund, or the department for infectious diseases of general hospitals in Malmö, Växjö and Hälsingborg. The diagnosis of acute virus meningoencephalitis was based on the following clinical and pathologic findings

1. Acute attack of headache, as a rule associated with neck-stiffness, dizziness, nausea and vomiting
2. Body temperature above 38° C for at least 3 days.
3. Increased number of cells in the cerebrospinal fluid, i.e. at least 5 cells per cu.mm.

Patients with any disease of the C.N.S. in their histories were not included. Neither were patients with any coexisting disease. Only cases in which the samples and the analysis were satisfactory were accepted. The material consisted of 131 persons who were examined during the first or second week of the disease. The sex and age distribution is given in Table 2

As in most meningoencephalitis series (WICKMAN 1907, FANCONI 1945, KILHAM 1949, STRÖM 1956, HOLMGREN et al. 1959) males were more common than females (62.6 % against 37.4 %) This difference was also roughly the same in the two age groups. In the group with mumps the proportion of males was still larger namely 73.2 %

The material examined fell into the aetiological groups in Table 3.

Of the 41 cases diagnosed as mumps, swelling of the parotid glands was not demonstrable in 8. In 6 of these, however the diagnosis was confirmed by a positive complement fixation test, in which the titre was increased fourfold. The

Department of Bacteriology University of Lund.

Table 2 Classification of patients according to sex and age at onset of disease

Sex and age in years		N	Per cent
Males	<15	24	18.3
„	>15	58	44.3
Females	<15	12	9.2
„	>15	37	28.2

Table 3 Composition of material at c

Mumps
Russian-Spring-Summer Encephalitis
Enterovirus infection
Adenovirus infection
Influenza
Herpes zoster
Measles
German measles
Virus not determined

remaining 2 patients had co-existing bilateral orchitis. The diagnosis of Russian-Spring-Summer Encephalitis was based on serological analysis. The complement fixation test showed a fourfold increase of the titre in 5 cases. In 1 case the titre was high in the first test, which was performed 1 week after the onset of the disease. The diagnosis of enterovirus was based on the demonstration of the virus in the faeces. The virus found in the 6 cases were Echo 3, Echo 6 Echo 9 Coxsackie B 5 Coxsackie A 8 and polio 1 virus, respectively Serological examination confirmed the diagnosis. The diagnoses of infections with adenovirus and influenza virus were based on the complement fixation test. The titre increase was fourfold throughout. Measles and German measles were diagnosed on the basis of the patient s history and data supplied by the doctor referring the patient to hospital and on the knowledge of the epidemiological situation.

In 70 i.e more than half of the patients the aetiology of the infection was not determined. Attempts were made to isolate the virus in 58 of the 70 cases in which the causal agent could not be identified. Samples were often sent to more than one laboratory In many cases 3 samples of the faeces were analysed. The 58 were also investigated serologically as is apparent from the following table. All the analysis were performed by routine methods. In the remaining 12 the patients were studied neither virologically nor serologically

Thanks to recent advances in virology and serology the frequency of cases of unknown causal agent has successively decreased, in one series down to 25 %

Central Bacteriological Laboratory of Stockholm City Stockholm.
State Bacteriological Laboratory Stockholm.
Virus Laboratory Malmö general hospital.

(Meyer et al. 1960) According to Macrae (1961) however such a favourable figure can only be obtained in cases examined during an epidemic. Of 119 persons examined in the present investigation, a definite diagnosis of the type of meningoencephalitis was made in 61 (51 %) Most of the diagnoses were, how ever made serologically for attempts to isolate the virus were successful in only 6 cases. In all 6 the virus was found in the faeces. In these cases the antibody titre was significantly increased and the virus found was therefore held responsible for the disease.

A fair proportion (31 %) of the cases consisted of mumps meningoencephalitis. The frequency of complicating disorders of the C.N.S. in mumps varies with the diagnostic criteria used and the frequency of examination of the C.S.F The figures on record range from 10 % (Enders 1959) to 65 % (Bang and Bang 1943) Mumps is so common that mumps meningoencephalitis represents a large group in several meningoencephalitis series, particularly in Scandinavian collections. The proportion in the present material, almost 25 % agrees well with that found in other Swedish series (Ström 1956, Widell 1958)

The finding of RSSE-virus infection only in persons above 20 years is consistent with the corresponding figure reported from Stockholm (Holmgren et al. 1959) In 1 case the increase in the titre was not fourfold, but the antibody titre on admission was high and persisted at that level. This case was therefore most likely also an instance of RSSE-infection, for antibody titres are rarely positive in a normal Swedish population (Svedmyr et al. 1958)

The material included only 3 cases of meningoencephalitis in association with German measles and measles, the complication being uncommon in these infections.

Table 4 Negative serologic tests in 58 cases of meningoencephalitis of undetermined virologic type

Complement-fixation test	mumps virus	4
	adenovirus	36
	Russian-Spring-Summer Encephalitisvirus	29
	influenzavirus	16
	poliomyelitisvirus	8
	and agglutination test listeria monocytogenes	9
	and dye test toxoplasmosis	6
Agglutination test	leptospira icterohaemorrhagica	7
Paul-Bunnell test		8
Cold haemagglutination test		16

CLINICAL PICTURE

Inflammation of the C.N.S., whatever its cause, can produce a number of relatively characteristic symptoms. *Symptoms of meningitis* are headache, vomiting and neck stiffness. Encephalitic processes can cause disturbances of consciousness and neurological signs of irritation and loss of sensory and motor function. In meningoencephalitis the symptoms of *meningitis* and *encephalitis* generally coexist with changing preponderance and severity of one or more of them.

The cases were classified as mild or severe according to the symptoms and signs noted. Most patients were examined by the author. Only in a *few* instances was classification based on the patient's history sheets. The following criteria were used.

Mild cases Largely unaffected general physical condition without neurological symptoms or signs of motor and sensory loss or irritation. Slight to moderate symptoms of meningitis—headache and nausea with or without neck-stiffness—for less than 5 days. Pyrexia (>38° C) for more than 3 days.

Severe cases Deteriorated general condition with lowered level of consciousness. Occurrence of other neurological signs. Severe or prolonged symptoms of meningitis, i.e. more than 5 days. Obstinate pyrexia, usually for 10—14 days.

Of the 131 cases, 89 (68 %) were classified as mild and 42 (32 %) as severe. Of the 42 severe cases consciousness was impaired in 31, facial paresis was noted in 3, pareses of the limbs in 3 and of the bladder in 1, and Babinski's sign in 1. In 9 cases pyrexia was obstinate. The fever was often accompanied by neck stiffness. In 4 patients the clinical picture was dominated by severe symptoms of meningitis. Some patients had more than one of these symptoms. The cases are grouped according to severity in Table 5, in which the group with mumps is given separately.

It is clear from the table that mild cases were much more common in the mumps group. The difference was almost significant.

In Table 6 the patients are grouped according to sex, age and severity of the symptoms.

The distribution of the patients according to severity of symptoms at onset of disease was somewhat uneven. On comparison between the sexes irrespective of age no substantial difference was noted. The number of males with mild

Conventional statistical methods were used (Hill, A. B. Principles of medical statistics, Lancet, London 1961)

Table 5 Classification of patients according to severity of symptoms at onset of disease

Diagnosis	Mild		Severe	
	N	Per cent	N	Per cent
Mumps	34	83	7	17
Miscellaneous	53	6	35	39

Table 6 Classification of patients according to sex age, and severity of symptoms at onset of disease

Sex and age in years	Mild		Severe	
	N	Per cent	N	Per cent
Males < 5		88	3	
> 5	38	66	20	34
Females < 5	8	67	4	33
> 5	22	60	15	40

Table 7 Classification of patients according to severity of symptoms and interval between onset of disease

Interval between onset of disease and admission	Mild		Severe	
	N	Per cent	N	Per cent
—3 days	66	72	6	28
4—7	20	65		35
8— 4	3	38	5	62

symptoms was 72 % compared with 61 % of the females. But the frequency of mild cases did tend to vary with age, the proportion of mild cases in patients below 15 years being 81 % against 63 % in the patients above this age limit. It is clear from the table that this difference was due mainly to the higher frequency of mild symptoms in the males below 15 years.

A similar distribution with a higher frequency of the milder form of the disease in children than in adults has been shown for poliomyelitis (FLAUM 1940) and for virus meningoencephalitis (MÜLLER and NYLANDER 1958)

For various reasons the interval between the onset and admission to hospital varied, mainly between 1 and 7 days (Table 7)

The ratio between the number of mild and severe cases was largely the same in the first two groups but very different in the third group, i.e. patients who had been ill for 8 to 14 days. In this group the proportion of severe cases was larger. It consisted of 3 persons who despite improvement were admitted for observation and of 5 persons who had not improved and were ill on admission to hospital.

CELLS IN C.S.F

According to most workers in this field the total number of cells does not vary significantly with the severity of meningoencephalitis (FANCONI 1945, PETTE 1954, BEDJANIC et al. 1955, v. OLDERSHAUSEN 1957, BREWIS 1961). That a normal number of cells in C.S.F. does not exclude viral invasion of the C.N.S. is known (WENNER 1959, SCHMIDT 1960, v. OLDERSHAUSEN 1961). TE WEN CHANG and WEINSTEIN (1962) even found the frequency of recovery of Echo 9-virus from the spinal fluid of patients with aseptic meningitis to be inversely related to the number of cells present in cases in which neutralizing antibodies could not be demonstrated.

The present material was divided into 4 groups according to the number of cells in the C.S.F.

One patient in whom the number of cells was normal at the time of admission was included because the clinical course of his symptoms was typical of meningoencephalitis and because the electro-encephalogram showed typical abnormalities. Somewhat more than half of all the patients had more than 100 cells per cu.mm. Only 7 % had more than 500 cells per cu.mm. The highest value noted was 970 cells per cu.mm. In Table 9 the severity of the symptoms is related to the degree of pleocytosis.

It is clear from the table that, as expected, the number of cells did not vary substantially with the severity of the disease.

Table 8 Classification of patients according to cell content of C.S.F. (cells per cu.mm.) at onset of disease

Cells per cu. mm.	N	Per cent
<5	1	
5—100	67	51
101—500	54	41
>500	9	7

Table 9 Cell content (cells per cu.mm.) of C.S.F in relation to severity of symptoms at onset of disease

Cells per cu. mm.	Mild		Severe	
	N	Per cent	N	Per cent
<5	1			
5—100	43	64	24	36
—500	39	72	5	28
>500	6	67	3	33

Table Total protein content (mg. per 00 ml.) in relation to severity of symptoms at onset of disease

Total protein in mg per 00 ml.	Mild		Severe	
	N	Per cent	N	Per cent
≤50	38	88	5	
5 — 00	45	63	27	37
> 00	6	38	1	62

TOTAL PROTEIN CONTENT

In the majority of series on record the concentration of the total protein in the C.S.F in virus meningoencephalitis is normal in 30—50 % and only moderately increased in the remainder (v. OLDERSHAUSEN 1957 BREWIS 1961 KAHLMETER 1961 LEPOW et al. 1962) Values above 100 mg. per 100 ml. are relatively rare. According to FARQUHAR (1945) the total protein content is often normal at the onset of the disease and increases successively *during the first week*, after which it gradually returns to normal. KILHAM (1949) *found that* in mumps meningoencephalitis the total protein content reached a peak later than did pleocytosis. In a series of 20 patients with mumps meningoencephalitis and 21 with lymphocytic choriomeningitis, who were examined by repeated puncture ADAIR et al. (1953) were unable to *demonstrate* a decrease of the average value of the total protein content during the first 2 weeks of the disease.

In an attempt to find out whether the total protein content is related *to the* severity of the disease, the present material was divided into 3 groups (Table 10)

In almost one third of the patients the total protein content was normal, and in almost 90 % it was less than 100 mg. per 100 ml. In 3 patients the concentra-

Table 11 Cell content (cells per cu.mm.) in relation to total protein content (mg. per 100 ml.) of C.S.F at onset of disease

Cell per cu. mm.	Total protein in mg per 100 ml.		
	≤ 50	51—100	> 100
<5	1		
5—100	16	37	4
101—500	16	30	8
>500		5	4

tion was more than 200 mg per 100 ml. The group in which the total protein content was normal consisted mainly (88 %) of patients with mild symptoms, while the group in which the total protein content was increased consisted mainly of severely ill patients. The correlation between the severity of the disease and the total protein content was highly significant. The possibility of a correlation between the total protein concentration and the clinical picture has received little attention in the literature. FANCONI (1945) stressed that in patients with poliomyelitis with bulbar and encephalitic symptoms the concentration of the total protein is lower than in those with spinal and thecal forms. WIDELL (1958) however, found that in adults with types of meningoencephalitis other than mumps meningoencephalitis, the total protein content of the C.S.F was higher in the severe cases than in the mild ones. BREWIS (1961) found no such correlation in poliomyelitis.

The number of cells is related to the total protein content of the C.S F found in the present material in Table 11

It is clear from Table 11 that counts below 100 cells per cu.mm. were most common in the patients in whom the total protein content of the C.S F was normal. The difference was, however, small and not significant.

IMMUNOELECTROPHORETIC STUDIES

The results of immunoelectrophoretic examination of the C.S.F in 131 persons grouped according to sex, age severity of disease time of lumbar puncture, number of cells and total protein content of the C.S F are accounted for below Concurrent immunoelectrophoresis of the serum was done in almost all cases, but in none could any pathological precipitate be demonstrated. Of the C.S F

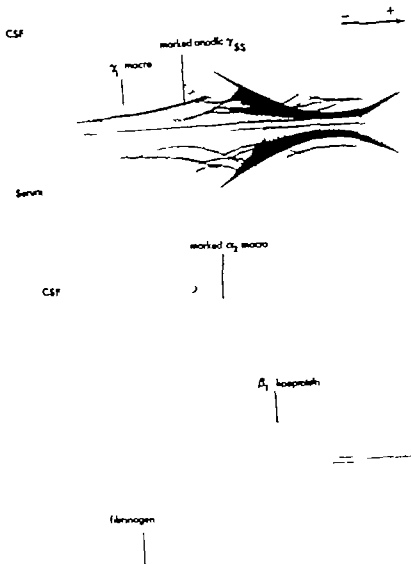


Fig. Immunoelectrophoretic pattern in C.S.F. and ... case B.L. No. 7 of case report ... anti-human-antiserum ... -macroglobulin and marked anodic ... -globulin ... demonstrated ... antiserum revealed ... marked α_2-macroglobulin precipitate. c. Sudan black showed β-lipoprotein precipitate ... rabbit-antihuman-antiserum. d. Antifibrinogen serum gave ... fibrinogen precipitate.



samples studied, a marked α_2-macroglobulin precipitate band was found in 67 β_1-lipoprotein in 50, fibrinogen in 75, γ_1-macroglobulin in 56 and a marked anodic γ_{2}-globulin precipitate in 38. In 22 cases the immunoelectrophoretic pattern was normal. The following illustrative case report may serve as an example of the clinical picture and immunoelectrophoretic findings.

B.L., male, aged 51 Hitherto healthy
Oct 24 1 25. Catarrhal symptoms and fever (38 C)
Nov 3. Fever (≤40° C) headache, nausea and giddiness.
Nov. 7 Admitted to hospital because of persistent symptoms. Lowered level of consciousness. Temp. 40 C.
Neck-stiffness. Otherwise normal.

Examination on admission Hb. 11.9 g. per 100 ml. RBC 3.9 mill. WBC 8,800. Diff count. N 74 %, E 1 %, B nil, L 24 %, M 1 %. E.S.R. 29 mm/1 hr.
Culture of faeces for virus gave no growth. Complement fixation test for influenza and ornithosis negative. Cold agglutination test negative.

Nov 10. E.E.G. showed diffuse signs of theta activity equal on both sides.
Nov. 17 Ophthalmologically normal.
Nov. 1 Nystagmography showed a pathologic change, which was, however not characteristic of encephalitis.
Nov 23 Afebrile.
Dec. 5. E.E.G. normal.
Dec. 15. Patient sent home.
Feb. 8. Re-examined and found to be healthy

Cerebrospinal fluid findings

Date	Cells per cu.mm.	Total protein mg. per 100 ml.	globulins in CSF in paper electrophoresis	marked α_2-macroglobulin	β_1-lipoprotein	fibrinogen	γ_1-macroglobulin	marked anodic γ_2-globulin
7/	74	64	normal	+	+	+	+	+
23/ 1	56	98	22.6 %	+	+		+	+
5/ 1	5	60	3.0 %		+		+	+
6/		44					+	+

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS SEX AND AGE

It is clear from Table 12 that the *marked* α_2-*macroglobulin* precipitate was almost equally common in both sex groups. An almost significant difference was, however found between the age groups, a marked α_2-macroglobulin pre-

Table 12 Classification of patients according to sex, age, and immunoelectrophoretic findings at onset of disease

Sex and age		N	marked α_2-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_M-globulin	
			N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
Females	<15	24	15	63	7	29	12	50	7	29	1	4
"	>15	58	29	50	23	40	37	64	25	43	22	38
Males	<15	12	9	75	5	41	7	58	8	67	2	17
"	>15	37	14	38	15	40	19	51	16	43	13	35

cipitate being demonstrable in 67 % of the lower and only 44 % of the higher age group. As to the *β_1-lipoprotein* precipitate, it was demonstrated in only 29 % of the boys, against about 40 % in the remaining patients. The difference was, however not statistically significant. *Fibrinogen* precipitate was the commonest finding and was demonstrated in 75 cases. The distribution among the groups was relatively even. It was seen in 64 % of the males above 15 years and in 50 to 58 % in the remaining three groups. The difference was not statistically significant. *γ_1-macroglobulin* precipitate was demonstrated in 56 cases, and its distribution among the groups was uneven. It was found in only 29 % of the boys, against 67 % of the girls, who were, however too few to allow testing of the difference statistically. γ_1-macroglobulin was equally common in both sexes above 15 years, namely 43 % and in both age groups. A *marked anodic γ_M-globulin* precipitate was the most uncommon finding and could be demonstrated in 29 % of the cases. It was seen in only 3 of the 36 patients below 15 years against 48 % of those above this age. The difference was significant.

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS AND SEVERITY OF SYMPTOMS

The frequencies of various immunoelectrophoretic findings in the 89 mild and the 42 severe cases are given in Table 13

It is apparent from Table 13 that a *marked α_2-macroglobulin* precipitate was demonstrated in 47 % of the mild cases against 60 % of the severe ones. The difference was not statistically significant. As to the frequency of the *β_1-lipoprotein* precipitate, the difference between the two groups was larger namely 29 % in the mild cases against 57 % in the severe ones. This difference was statistically significant. A *fibrinogen* precipitate was demonstrated in 54 % of

Table 13 Classification of patients according to severity of symptoms at onset of disease and immunoelectrophoretic findings

Clinical symptoms	N	marked α_2-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_2-globulin	
		N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
Mild	89	42	47	26	29	48	54	33	37	20	22
Severe	4	5	60	24	57	7	64	13	55	8	43

Table 14 Classification of patients according to interval between onset of symptoms and lumbar puncture and the immunoelectrophoretic findings

Day of disease	N	marked α_2-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_2-globulin	
		N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
— 3	92	48	52	34	37	52	57	39	42	1	3
4— 7	3	6	52	5	48		68	14	45	2	39
8— 4	8	3	38		3		5	3	38	5	63

the mild cases and in 64 % of the severe ones. The difference was not statistically significant. A γ_1-*macroglobulin* precipitate was noted in 37 % of the mild cases and 55 % of the severe ones. The difference was almost significant. A *marked anodic* γ_2-*globulin* precipitate was found in 22 % of the mild cases and in 43 % of the severe ones. The difference was almost significant.

CORRELATION BETWEEN THE INTERVAL BETWEEN ONSET OF DISEASE AND LUMBAR PUNCTURE AND THE IMMUNOELECTROPHORETIC FINDINGS

As mentioned previously lumbar puncture was invariably done on the day of admission. For various reasons the interval between the onset of the symptoms and admission varied. The patients are grouped according to this interval in Table 14.

The table shows that a *marked* α_2-*macroglobulin* precipitate was demonstrated in somewhat more than half of the 123 patients examined during the first week. Of the 8 patients admitted during the second week of the disease, the component was found in only 3, i.e. 38 %. A β_1-*lipoprotein* precipitate was demonstrated in 37 % of the patients admitted within the first 3 days of the disease,

in 48 % of the 31 persons admitted a few days later and in only 1 of the 8 admitted during the second week of the disease. A *fibrinogen* precipitate was noted in 57 % of the patients admitted within the first few days of the disease. Of those who had been cared for a few further days at home, the precipitate was seen in 68 % against only 25 % of those admitted in the second week of the disease. The frequency of the fractions thus tended to decrease during the second week of the disease.

A γ_1-*macroglobulin* precipitate was demonstrated in 42 % of all the patients examined on admission. The frequency of the fraction did not vary substantially with the interval between the onset of symptoms and admission to hospital. The *marked anodic* γ_m-*globulin* precipitate showed an increasing tendency. Of those patients examined within the first 3 days, in the latter part of the first week, and in the second week, respectively, it was found in 23 %, 39 % and 63 %. The frequency of a marked anodic γ_m-globulin precipitate among patients examined in the second week was significantly higher than that noted for those examined in the first week.

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS, CELL COUNT AND THE TOTAL PROTEIN CONTENT

As mentioned previously, a correlation was found between the clinical picture and the total protein content but not with the degree of pleocytosis. The following 5 tables give the correlations found between the number of precipitates demonstrated, the number of cells and the total protein content. Since the number of cells tended to vary with the total protein content, the results are given in the form of a cross-table. This shows the correlation with the number of cells within each range of concentration of the total proteins as well as the correlation between the total protein concentration in the different cell-count ranges.

Table 15 shows that *marked* α_2-*macroglobulin* precipitate was demonstrated in all together 67 persons. It was found in 35 % of those with less than 100 cells per cu.mm., in 67 % of those who had between 101 and 500 cells per cu. mm. and in 78 % of those who had more than 500 cells per cu.mm. This increase in the frequency of the protein with the cell count was highly significant.

A marked α_2-macroglobulin precipitate was demonstrated in 42 % of those with a normal total protein content, in 51 % of those who had between 51 and 100 mg per 100 ml. and in 75 % of patients with still higher concentrations. This tendency of the frequency of marked α_2-macroglobulin precipitate to increase with the total protein content was not so strong as the correlation with

Table 5 Marked α_2-macroglobulin precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg. per 00 ml.) at onset of disease

Total protein in mg. per 00 ml.	≤ 50		5 — 00		> 00		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5							
5— 00	26	7	37	4	4	3	25
1 1—500	6		30	19	8	6	67
>500			5	4	4	3	78
Per cent		42		5		73	

Table 6 β-lipoprotein precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg. per 00 ml.) at onset of disease

Total protein in mg. per 00 ml.	≤ 50		5 — 00		> 00		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5							
5— 00	26	5	37	1	4		28
—500	6	1	30	7	8	4	44
>500			5	3	4	4	78
Per cent				44		56	

pleocytosis, and was partly due to the fact that patients with a cell count of less *than 100 per cu.mm. more often had* a normal total protein content than in the other groups.

It is clear from Table 16 that a *β_1-lipoprotein* precipitate was demonstrated in 50 persons. It was found in 28 % of 68 persons with a cell count of less than 100 per cu.mm., in 44 % of those with a count of 101 to 500 cells per cu.mm., and in 78 % of those with a still higher cell count. This increase in the frequency of a β_1-lipoprotein precipitate with increasing cell count was significant like that of a α_2-macroglobulin precipitate.

The relation with the total protein content is illustrated by the following figures 21 % of the patients with a normal total protein content showed a β_1-lipoprotein precipitate, as did 44 % of those with 51 to 100 mg. per 100 ml. and 56 % of those with a still larger total protein content. This correlation *i.e.* that patients with a larger total protein content more often showed a β_1-lipopro-

Table 17 Fibrinogen precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg. per 100 ml.) at onset of disease

Total protein in mg. per 100 ml.	≤ 50		51—100		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1	1					
5—100	26	14	37	16	4	2	*49*
101—500	16	8	30	22	8	4	*63*
>500			5	4	4	4	*89*
Per cent		*53*		*58*		*63*	

Table 18 γ-macroglobulin precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg per 100 ml.) at onset of disease

Total protein in mg per 100 ml.	≤ 50		51—100		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1	1					
5—100	26	4	37	11	4	4	*29*
101—500	16	8	30	18	8	4	*56*
>500			5	3	4	3	*67*
Per cent		*30*		*44*		*69*	

Table 19 Marked anodic γ_m-globulin precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg per 100 ml.) at onset of disease

Total protein in mg per 100 ml.	≤ 50		51— 00		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1						
5—100	26	3	37	9	4	2	*21*
101—500	16	2	30	12	8	6	*37*
>500			5	1	4	3	*44*
Per cent		*12*		*31*		*68*	

tein precipitate than those with a smaller total protein content, was almost statistically significant and thereby differed from the results found for α_2-macroglobulin.

Table 17 shows that *fibrinogen* was found in the C.S.F. of 75 persons and was thus the commonest finding. Of the patients with a lower cell count than 100 per cu.mm., 49 % showed this precipitate. A fibrinogen precipitate was still more common, or in 63 % of those with 101—500 cells per cu.mm., and in as many as 89 % of those with higher cell counts. The correlation between the frequency of fibrinogen and the degree of pleocytosis was almost significant. The tendency was thus the same as for α_2-macroglobulin and β_1-lipoprotein, but less pronounced.

The tendency to an increased frequency of precipitates in those with a large total protein content was not so marked. Thus, a fibrinogen precipitate was demonstrated in 53 % of the patients with a normal total protein concentration, in 58 % of those with a concentration of 51 to 100 mg. per 100 ml. and in 63 % of those with still higher concentrations.

Table 18 shows the frequency with which a *γ-macroglobulin* precipitate was demonstrated in 56 patients. The precipitate was thus more common than a marked anodic γ_m-globulin precipitate. Of those patients in whom the count did not exceed 100 cells per cu.mm., a γ_1-macroglobulin precipitate was found in 29 %. A γ_1-macroglobulin precipitate was demonstrated in 56 % of those with a cell count of 101—500 cells per cu.mm. and in 67 % of those with still more severe pleocytosis. Like the 3 preceding proteins, its frequency increased with the cell count. The correlation was significant.

The correlation with the total protein content is clear from the fact that 30 % of those with a normal content were positive, against 44 % of those with a concentration between 51 and 100 mg. per 100 ml. and 69 % of those with still higher concentration. This correlation was almost significant, like that found for β_1-lipoprotein.

The table shows that a *marked anodic γ_m-globulin* precipitate was found in only 38 patients and was thus the least common finding. Of the patients who had a lower cell count than 100 per cu.mm., 21 % showed a marked anodic γ_m-globulin precipitate compared with 37 % of those with 101 to 500 cells and 44 % of those with still higher cell counts. In contrast to the previously mentioned proteins, the tendency of a marked anodic γ_m-globulin precipitate to be more common when the cell count was high was not strong.

On the other hand, the correlation between a marked anodic γ_m-globulin precipitate and the total protein content was highly significant and was stronger

Table 17 Fibrinogen precipitate in relation to cell content (*cells per cu.mm.*) and total protein content (mg per 100 ml.) at onset of disease

Total protein in mg per 100 ml.	≤ 50		51—100		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1	1					
5—100	26	14	37	16	4	2	49
101—500	16	8	30	22	8	4	63
>500			5	4	4	4	89
Per cent		53		58		63	

Table 18 γ macroglobulin precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg per 100 ml.) at onset of disease

Total protein in mg. per 100 ml.	≤ 50		51—100		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1	1					
5—100	26	4	37	11	4	4	29
101—500	16	8	30	18	8	4	56
>500			5	3	4	3	67
Per cent		30		44		69	

Table 19 Marked anodic γ_M-globulin precipitate in relation to cell content (cells per cu.mm.) and total protein content (mg. per 100 ml.) at onset of disease

Total protein in mg per 100 ml.	≤ 50		5 — 00		> 100		
Cells per cu.mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	1						
5—100	26	3	37	9	4	2	21
101—500	16	2	30	12	8	6	37
>500			5	1	4	3	44
Per cent		12		31		68	

tein precipitate than those with a smaller total protein content, was almost statistically significant and thereby differed from the results found for α_2-macroglobulin.

Table 17 shows that *fibrinogen* was found in the C.S.F. of 75 persons and was thus the commonest finding. Of the patients with a lower cell count than 100 per cu.mm., 49 % showed this precipitate. A fibrinogen precipitate was still more common, or in 63 % of those with 101—500 cells per cu.mm., and in as many as 89 % of those with higher cell counts. The correlation between the frequency of fibrinogen and the degree of pleocytosis was almost significant. The tendency was thus the same as for α_2-macroglobulin and β_1-lipoprotein, but less pronounced.

The tendency to an increased frequency of precipitates in those with a large total protein content was not so marked. Thus, a fibrinogen precipitate was demonstrated in 53 % of the patients with a normal total protein concentration, in 58 % of those with a concentration of 51 to 100 mg. per 100 ml. and in 63 % of those with still higher concentrations.

Table 18 shows the frequency with which a *γ_1-macroglobulin* precipitate was demonstrated in 56 patients. The precipitate was thus more common than a marked anodic γ_2-globulin precipitate. Of those patients in whom the count did not exceed 100 cells per cu.mm., a γ_1-macroglobulin precipitate was found in 29 %. A γ_1-macroglobulin precipitate was demonstrated in 56 % of those with a cell count of 101—500 cells per cu.mm. and in 67 % of those with still more severe pleocytosis. Like the 3 preceding proteins, its frequency increased with the cell count. The correlation was significant.

The correlation with the total protein content is clear from the fact that 30 % of those with a normal content were positive, against 44 % of those with a concentration between 51 and 100 mg. per 100 ml. and 69 % of those with still higher concentration. This correlation was almost significant, like that found for β_1-lipoprotein.

The table shows that a *marked anodic γ_2-globulin* precipitate was found in only 38 patients and was thus the least common finding. Of the patients who had a lower cell count than 100 per cu.mm., 21 % showed a marked anodic γ-globulin precipitate compared with 37 % of those with *101 to 500 cells* and 44 % of those with still higher cell counts. In contrast to the previously mentioned proteins, the tendency of a marked anodic γ_2-globulin precipitate to be more common when the cell count was high was not strong.

On the other hand, the correlation between a marked anodic γ_2-globulin precipitate and the total protein content was highly significant and was stronger

than that found for any of the other 4 proteins. Only 12 % of the patients with a normal total protein concentration showed a marked anodic γ_{m}-globulin precipitate against 31 % of those with 51 to 100 mg per 100 ml. and 68 % of those with still higher concentrations.

CORRELATION BETWEEN VIROLOGIC DIAGNOSIS AND IMMUNOELECTROPHORETIC FINDINGS

A virological diagnosis was obtained in 61 (46.5 %) of the patients. Of these, 41 had mumps meningoencephalitis. This was the only group large enough to warrant statistical evaluation. The remaining 20 cases represented 7 different diagnoses.

The two groups did not differ in respect of the frequency of marked α_2-macroglobulin precipitate or fibrinogen. Some differences were, however found between the groups in respect of β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_{m}-globulin precipitates. These differences were, however not significant.

The immunoelectrophoretic findings in the 20 cases in the miscellaneous group are listed in Table 21

Of the 6 cases of RSSE with a total protein concentration of 57 to 118 mg. per 100 ml. (average 92.7 mg per 100 ml.) marked α_2-macroglobulin precipitate was seen in 4, β_1-lipoprotein in 5 fibrinogen in 4, γ_1-macroglobulin in 5 and marked anodic γ_{m}-globulin precipitate in 4.

In those patients in whom enterovirus was the causal agent the total protein concentrations ranged between 40 and 110 mg per 100 ml. (average 59.8 mg per 100 ml.) In 2 of them the protein pattern was normal. Of the others, marked α_2-macroglobulin precipitate and fibrinogen were noted in 2 γ_1-macroglobulin in 1 and marked anodic γ_{m}-globulin precipitate in 1

The patients with influenza meningoencephalitis had a total protein concentration of 57 respectively 60 mg per 100 ml. and in both of them the immuno-

Table 20 Immunoelectrophoretic findings in patients grouped according to type of meningoencephalitis at onset of disease

Diagnosis	N	marked α_2-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_{m}-globulin	
		N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
Mumps	41	20	49	12	29	25	61	15	37	1	31
Miscellaneous	90	47	52	38	42	50	56	41	46	8	41

Table 2 Immunoelectrophoretic findings in 20 patients with known type of meningoencephalitis at onset of disease

Diagnosis	N	marked α_2-macro-globulin	β-lipo-protein	fibri-nogen	γ-macro-globulin	marked anodic γ_{2a}-globulin
RSSE	6	4	5	4	5	4
Enterovirus	6					
Adenovirus						1
Influenza						
Herpes zoster						
Measles						1
German measles						

electrophoretic pattern was normal. In both cases of adenovirus meningoencephalitis with a total protein concentration of 100 respectively 125 mg. per 100 ml. the C.S.F. contained β_1-lipoprotein. The other proteins demonstrated were found *in only 1 of them*. The patient with Herpes zoster and with a protein concentration of 60 mg. per 100 ml. showed a marked anodic γ_{2a}-globulin precipitate as the only pathologic finding.

The two patients with complications after measles and with a total protein concentration of 62 respectively 89 mg. per 100 ml. had marked α_2-macroglobulin precipitate and γ_1-macroglobulin as well as β_1-lipoprotein. Fibrinogen and a marked anodic γ_{2a}-globulin precipitate were noted in each of 2 cases. The patient with German measles meningoencephalitis and with a total protein concentration of 145 mg. per 100 ml. showed only a γ_1-macroglobulin precipitate.

DISCUSSION

According to Bowsher (1960) the C.S.F. formation should be seen as follows:

1. Substances pass by diffusion into the fluid from the plasma across the membranes (including ependyma) which line the C.S.F. containing spaces. The rate of entry is probably largely determined by particle size, but to some extent is also related to lipoid solubility: this latter on account of the identity of C.S.F. and extracellular fluid of the neuraxis.
2. Certain substances, in addition to entry by diffusion, are actively secreted into the C.S.F. by the choroid plexuses. Such substances probably include protein and almost certainly sodium.

3 Water can enter and leave the system freely at all points without let or hindrance and with great rapidity It will always enter in sufficient quantity to render the secreted sodium isotonic with the plasma."

No unanimity has so far been achieved as to whether the blood-cerebrospinal fluid barrier should be sought in the endothelium of the capillaries, the pia-glia, the astrocytic glia, the ependyma, the choroid plexus or in the arachnoid membranes (REFSUM 1963) The blood C.S F barrier is thus conceived *inter alia* as a semipermeable membrane, through which certain proteins, depending on their size and configuration pass from the plasma. It is known that in healthy persons the normally occurring proteins enter the C.S F-containing space at varying rates with consequent differences in their concentration there. FRICK and SCHEID-SEYDEL (1958 1958 a) who used ^{131}I marked albumin and globulin found that intravenously injected albumin reached equilibrium with the C.S.F after about 60 hours and that the corresponding time for globulin was about 100 hours. Immunochemical and electrophoretic studies have shown that in the normal C.S F albumin is present in a higher concentration than γ-globulins relative to plasma, suggesting selective ultrafiltration from plasma favouring the smaller molecule. FRICK (1963) also showed that the concentration of transferrin in normal C.S F is 6 7 rel. %, which means that the concentration of transferrin in the C.S F is one third higher than in the serum.

One of the purposes of the present study was to find out to what extent particularly large molecular proteins not normally occurring in the C.S F can be demonstrated in the C.S.F of patients with acute virus meningoencephalitis. The material consisted of 131 patients in whom the C.S F and, with but few exceptions, the serum were studied immunoelectrophoretically The following proteins were studied in the C.S F α_2-macroglobulin, β_1-lipoprotein, fibrinogen, γ_1-macro- and γ_m-globulin. The first 3 proteins are called transproteins to indicate a possible difference from the two immunoglobulins which may have been formed in the C.S F space. The commonest findings were fibrinogen and marked α_2-macroglobulin precipitate which were demonstrated in 57 % respectively 51 % of the patients. As mentioned previously fibrinogen and its decomposition products are immunologically identical. They can, however be identified because the decomposition products have a different rate of migration and often consist of 2—3 determinants. Only in 4 out of 75 cases was there reason to suspect that the antigen was not fibrinogen. γ-macroglobulin and β_1 lipoprotein precipitates were less common and occurred in 42 % respectively 38 % As mentioned, for an anodic γ_m-globulin precipitate to be classified as

marked, the band had not only to extend into the α_2-region but also to be marked. In this respect then the criteria differ from those of CLAUSEN et al. (1962) with the result that the findings are not strictly comparable.

In the light of the above-mentioned conception of the blood-C.S.F. barrier as a semipermeable membrane, it was but natural to expect the β_1-lipoprotein, which has the highest molecular weight, to occur least often in the C.S.F. This was also found to hold for β_1-lipoprotein by ROSENTHAL and SOOTHILL (1962) who, using gel diffusion, determined the concentration of certain proteins in the C.S.F. in controls and in persons with neurological diseases. On comparison with the α_2-macroglobulin and the β_1-lipoprotein they found that the latter protein occurred in the lowest concentration in the C.S.F. On the other hand, in an immunoelectrophoretic investigation SVENNERHOLM et al. (1961) found that β_1-lipoprotein could be demonstrated more frequently than the other large molecular proteins in patients with malignant tumours. β_1-lipoprotein did not occur often in the present material as the only pathological precipitate. Only in 2 persons was this noted. On the other hand a fibrinogen or a marked α_2-macroglobulin precipitate occurred more often as the only pathological finding which may be explained by the assumption that when the disorder of the blood-C.S.F. barrier is less severe, the barrier still prevents certain proteins from entering the C.S.F. space.

In the first stage of virus infection the 2 immunoglobulins tend to occur in the C.S.F. simultaneously with one or more of the 3 transproteins, presumably owing to a disturbance of the blood-C.S.F. barrier. But the γ_1-macroglobulin as well as the marked anodic γ_{ss}-globulin precipitate was demonstrated as the only pathological precipitate in the C.S.F. This was seen in 6 persons in whom the γ-globulin concentration in the serum, as estimated by paper electrophoresis, was normal except in 1 in whom it was slightly increased. The immunoglobulin precipitates in the serum were markedly increased. The question in these cases is whether the immunoglobulins came from the serum or whether they were formed intrathecally. Evidence is available that antibodies can be formed in the C.S.F. space in disease. CLAUSEN (1962) distinguishes different forms of barrier disorders. If the breakdown of blood-C.S.F. barrier is only partial α_2-macroglobulin and β_1-lipoprotein appear in the C.S.F., but the γ_1-macroglobulin is not increased in the absence of immunizing factors. If on the other hand, the C.N.S. is infected, the γ-globulin appears to be increased more markedly in relation to the other proteins in the C.S.F. BURTIN and POIGDALO (1954) described some cases of meningoencephalitis in which the γ-globulin concentration was increased as determined by an immunochemical method, but the *total* protein concentra-

3 Water can enter and leave the system freely at all points without let or hindrance and with great rapidity It will always enter in sufficient quantity to render the secreted sodium isotonic with the plasma."

No unanimity has so far been achieved as to whether the blood-cerebrospinal fluid barrier should be sought in the endothelium of the capillaries, the pia-glia, the astrocytic glia, the ependyma, the choroid plexus or in the arachnoid membranes (REFSUM 1963) The blood-C.S F barrier is thus conceived *inter alia* as a semipermeable membrane, through which certain proteins, depending on their size and configuration, pass from the plasma. It is known that in healthy persons the normally occurring proteins enter the C.S F -containing space at varying rates with consequent differences in their concentration there. FRICK and SCHEID-SEYDEL (1958 1958 a) who used ^{131}I-marked albumin and globulin found that intravenously injected albumin reached equilibrium with the C.S.F after about 60 hours and that the corresponding time for globulin was about 100 hours. Immunochemical and electrophoretic studies have shown that in the normal C.S F albumin is present in a higher concentration than γ-globulins relative to plasma, suggesting selective ultrafiltration from plasma favouring the smaller molecule. FRICK (1963) also showed that the concentration of transferrin in normal C.S F is 6 7 rel. % which means that the concentration of transferrin in the C.S F is one third higher than in the serum.

One of the purposes of the present study was to find out to what extent particularly large molecular proteins not normally occurring in the C.S F can be demonstrated in the C.S.F of patients with acute virus meningoencephalitis. The material consisted of 131 patients in whom the C.S F and, with but few exceptions, the serum were studied immunoelectrophoretically The following proteins were studied in the C.S F α_2-macroglobulin, β_1-lipoprotein fibrinogen, γ_1-macro- and γ_m-globulin. The first 3 proteins are called transproteins to indicate a possible difference from the two immunoglobulins which may have been formed in the C.S F space. The commonest findings were fibrinogen and marked α_2-macroglobulin precipitate which were demonstrated in 57 % respectively 51 % of the patients. As mentioned previously fibrinogen and its decomposition products are immunologically identical. They can, however be identified because the decomposition products have a different rate of migration and often consist of 2—3 determinants. Only in 4 out of 75 cases was there reason to suspect that the antigen was not fibrinogen. γ -macroglobulin and β_1 lipoprotein precipitates were less common and occurred in 42 % respectively 38 % As mentioned for an anodic γ_m-globulin precipitate to be classified as

present material 43 patients were included who had a normal total protein concentration, i.e. up to 50 mg. per 100 ml., though some of them were severely ill. In some cases, however a substantial increase from a low normal value to 50 mg per 100 ml. might have occurred, particularly in the children. This and the sensitivity of the method, which allows measurement of micrograms, should be borne in mind in the evaluation of the finding that of 43 patients in whom the total protein content of the C.S.F. was normal at the onset of the disease, a marked α_2-macroglobulin precipitate was seen in as many as 18, β_1-lipoprotein in 9, fibrinogen in 23, γ_1-macroglobulin in 13 and a marked anodic γ_{ss}-globulin-precipitate in 5. Yet the occurrence of certain large molecular proteins in the C.S.F. without a simultaneous increase of the normally occurring proteins was noteworthy. It is therefore also understandable that large molecular proteins were most common among patients with a high total protein concentration. This tendency however varied from one protein to another. Concerning the 2 commonest findings, a marked α_2-macroglobulin precipitate and fibrinogen, the tendency was not significant. For β_1-lipoprotein and γ_1-macroglobulin the correlation was almost significant. For γ_{ss}-globulin the difference was greatest. This precipitate was marked in only 23 % of the patients who had less than 100 mg per 100 ml. but in as many as 70 % of those with a higher concentration of the total protein.

In the present series, as in earlier materials, the order of the increase of the cell count in the C.S.F. was not correlated with the severity of the disease. In some of the most severely ill patients, in whom the encephalitic components were predominant, the cell increase was only slight. On the other hand, a correlation was found between the degree of pleocytosis and the frequency of precipitates of each of the 5 proteins studied. For α_2-macroglobulin the correlation was highly significant, in that patients with a high cell count had a relatively larger number of positive findings than the others. A significant correlation was demonstrated between the frequency of β_1-lipoprotein respectively γ_1-macroglobulin bands and the degree of pleocytosis. The corresponding correlation with fibrinogen was almost significant. The correlation between a marked anodic γ_{ss}-globulin precipitate and the cell count was not close.

On investigation of the correlation between the manifestations of the disease and the frequency of precipitates it was found that the frequency of each of the proteins studied tended to vary with the severity of the clinical picture. This correlation was strongest for the two immunoglobulins and the β_1-lipoproteins.

The material included 41 persons with mumps meningoencephalitis. Of the remaining 90 patients, a firm virologic diagnosis was made in 20. The frequency

tion of C.S.F was normal. FRICK and SCHEID-SEYDEL (1958 a) who used ¹³¹I marked γ-globulin found that patients with infections of the C.N.S had γ-globulin that had passed from the serum as well as "liquoreigenes" which was occasionally as much as 90 % of the total γ-globulin in the C.S.F MORGAN (cit. HUMPHREY and WHITE 1963) injected poliomyelitis virus intracerebrally into monkeys and found the highest antibody titre in the body within the anterior horn of the spinal cord and little or no antibody in the blood In neurosyphilis the Wassermann reaction may be positive in the C.S.F and negative in the serum, and in trypanosoma gabiense MATTERN (1962) showed that the concentration of the γ_1-macroglobulin as judged by immunoelectrophoresis, was much lower in the serum than in the C.S F and that after adequate treatment of the disease the γ_1-macroglobulin concentration in the C.S.F., but not in the blood, persisted According to BOWSHER (1960) "it is now possible to state with confidence that the leptomeninx fulfils the 3 criteria currently considered essential for a tissue to be regarded as reticuloendothelial

1 It takes up vital dyes. (GOLDMAN 1913)
2 It contains reticulin, as shown by special stains (MILLEN and WOOLLAM 1954 a)
3 It shows haemopoietic activity in the embryo (ARIËNS KAPPERS 1958)

One might also imagine that antibodies are formed by round cells occurring in the blood, and that these cells can pass out into the tissue and establish them selves as histiocytes and fibroblasts (cit. HITZIG 1963)

Characteristic of a viral attack of the central nervous system is an engorgement of vessels down to capillary level, which may persist for several days after in fection Often associated with the hyperaemia is an escape of plasmatic fluid (HAYMAKER et al. 1958) In the presence of this more or less severe breakdown of the blood C.S F barrier the total protein content of the C.S.F increases. The degree of such an increase in the present material was found to be correlated with the clinical severity of the disease. From examinations with paperelectrophoresis we know that during the first phase of a meningoencephalitis the relative concentration of the albumin in the C.S F increases and exceeds that in the plasma (MATIAR and SCHMIDT 1957 WIDELL 1958) The increase of the total protein content of the C.S F is due mainly to the proteins with a lower molecular weight and normally occurring in the C.S F

As mentioned previously one of the criteria of the diagnosis of meningoen cephalitis was pleocytosis but not an increase of the total protein content. In the

During this period, however a change appeared to have occurred with a reduction of the frequency of the β_1-lipoprotein and fibrinogen precipitates. The two macroglobulins were demonstrated with roughly equal frequency irrespective of the week in which they were examined, but that of marked anodic γ_{ss}-globulin precipitate tended to increase. Of the 5 marked anodic γ_{ss}-globulin precipitates, 3 were found in patients who had been cared for at home during the first week and who had improved and had only slight symptoms on admission. The average protein concentration in these 3 patients was 64 mg. per 100 ml. The remaining 2 precipitates were found in 2 out of 5 persons admitted after 7 10 days' fever with severe symptoms. The average protein concentration was 73.6 mg. per 100 ml. That the relative frequency of the immunoglobulins did not decrease may be due to active antibody formation, which particularly in virus infections often persists for several months. That the number of transproteins decreased may be regarded as a sign of healing of the barrier

SUMMARY

The investigation was carried out on 131 patients in the acute phase of virus meningoencephalitis. Aetiological diagnoses were made in 61 cases. On clinical grounds the material was divided into 2 groups. 68 % were classified as having a mild form of the disease. The clinical picture was less severe in the children. No correlation was found between the severity of the disease and the degree of pleocytosis. On the other hand, a significant correlation was found between the severity of the disease and the increase of the total protein content. The C.S.F was examined immunoelectrophoretically regarding 3 plasma proteins normally not occurring in the C.S.F and α_2-macroglobulin and γ_{ss}-globulin, which occur in low concentration. A marked α_2-macroglobulin precipitate was noted in all together 67 patients, β_1-lipoprotein in 50 fibrinogen in 75, γ_1-macroglobulin in 56, and marked anodic γ_{ss}-globulin precipitate in 38.

A marked anodic γ_{ss}-globulin precipitate was noted more *often in adults* and a marked α_2-macroglobulin precipitate more often in children. Otherwise no definite differences were noted with sex or age. α_2-macroglobulin and fibrinogen did not appear to vary with the severity of the disease. On the other hand, β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_{ss}-globulin were more common in the severe cases. Marked α_2-macroglobulin, β_1-lipoprotein, fibrinogen and γ_1-macroglobulin were demonstrated more often among patients with a high *cell*

of β_1 lipoprotein, marked anodic γ_m- and γ_1-macroglobulin precipitates in the C.S.F in the patients with mumps meningoencephalitis was lower than in the remainder and may be ascribed to their lower concentration of the total protein. It was also noteworthy that the clinical picture of the patients with mumps meningoencephalitis was less severe than that of the other patients. Thus the frequency of mild cases was almost significantly higher in this group than in the group with mixed virological aetiology

The numbers of the patients with other firm virological diagnoses were too small to allow comparison It was, however obvious that in patients with RSSE, in which the protein concentration was often high, several precipitates of both transprotein and immunoglobulin were demonstrated. Two patients with measles meningoencephalitis also showed evidence of severe disorder of the blood C.S F barrier with most of the pathological precipitates.

A marked anodic γ_m-globulin precipitate in the C.S.F was uncommon in children. Only 3 of those below 15 years, i e 8 % showed this fraction compared with 37 % of the adults. On comparison of the immunoelectrophoretic patterns of sera from adults and children it was also found that the adults more often showed a marked anodic γ_m-globulin precipitate. The present investigation also revealed a strong correlation between the total protein content and the frequency of a marked anodic γ_m-globulin. The fact that 55 % of the children had less than 50 mg per 100 ml. compared with 24 % of the adults may help to explain the difference. Paper electrophoresis also showed that the absolute γ-globulin concentration in the C.S F was much higher in adults than in children. In contrast to what was found for a marked anodic γ_m-globulin precipitate, a marked a_1-macroglobulin precipitate proved more common in the lower age group, namely 67 % against 45 % This precipitate was found most often in the boys. Since the cell count was on the average higher in the children than in the adults, the correlation between pleocytosis and the frequency of a marked a_1-macroglobulin precipitate could explain the disproportion of this protein within the various age groups. On closer examination of the relationship between age and the cell count it was, however found that, whatever the cell count, a marked a_1-macroglobulin precipitate was more common in the children. The correlations between the frequency of a marked a_1-macroglobulin precipitate and pleocytosis respectively age thus existed independently of one another

In most of the patients lumbar puncture was done within the first week of the disease. The frequency of positive findings was roughly equal on all days of that week. Only 8 persons were examined for the first time in the second week so that comparisons between the groups must be evaluated with caution.

Table 22 Classification of patients according to sex, age and severity of symptoms 2–3 weeks after onset of disease

Sex and age years		Convalescents		Still ill	
		N	Per cent	N	Per cent
Males	< 5	1	92		8
	> 5	15	7		[illegible]
Females	< 5		25	6	75
	> 5	4	67	7	33

Table 23 Classification of patients according to cell count (cells per cu.mm.) in C.S.F. and severity of symptoms 2—3 weeks after onset of disease

Cells per cu. mm.	Convalescents		Still ill	
	N	Per cent	N	Per cent
<5	3	79	4	
5— 00	35	7	5	50
1 1—500	3	58	5	6

mumps meningoencephalitis against 22 % of the aforementioned 77 patients in whom the C.S.F. was re-examined 2—3 weeks after the onset of the symptoms.

The sex and age distribution of the two groups is given in Table 22.

It is clear from the table that in the group above 15 years the percentage of patients with severe symptoms did not vary with sex. About 70 % of them had practically recovered by this time. The percentage was practically the same also for those below 15 years. But in the younger age group the clinical picture differed significantly with sex, for only one fourth of the girls could be regarded as convalescent, against practically all the boys.

CELLS

Pleocytosis usually reaches its peak within the first stage of the disease (FANCONI 1945). As therefore expected, the number of cells in the C.S.F. was lower than that found at onset on the disease (Table 23).

At this examination the cell count was normal in 19 and exceeded 100 cells per cu.mm in only 8.

The proportion of convalescents among those with a normal or moderately increased cell count was about twice as large as among those with a higher cell count. In other words, a correlation now began to appear between the severity of the disease and the number of cells in the C.S.F., which was not discernible on analysis of the values noted on admission of the patients.

TOTAL PROTEIN CONTENT

Like the cell count, the total protein content of the C.S.F. tended to become normal in this stage of the disease.

count. β_1-lipoprotein, γ_1-macro- and marked anodic γ_{sa}-globulin precipitate were demonstrated more frequently in patients with a high protein concentration. These 5 plasma protein precipitates were also often demonstrated in patients with a *normal protein concentration*

All of these proteins may have passed into the C.S F from plasma, but it cannot be excluded that immunoglobulins, γ macroglobulin and γ_{sa}-globulin, may have been formed in the *cerebrospinal fluid space, especially in cases where* the immunoglobulin were the only pathological precipitate found.

Examination 2—3 Weeks after Onset of Disease

MATERIAL

Most of the 131 patients with acute virus meningoencephalitis and described in the preceding chapter spent 2 weeks or more in hospital Only exceptionally did patients leave hospital earlier *and then they were re-examined at the outpatient* department 2—3 weeks after the onset of the symptoms. Thus, all of the 131 persons were examined 2—3 weeks after the initial clinical manifestation of the disease. The patients were divided into 2 groups according to the findings at this examination.

Convalescents who had no clinical symptoms and who felt well as long as they did not exert themselves.

Patients who still had symptoms mainly pareses, fever severe headache, nausea, *neck-stiffness, severe fatigue, and psychic symptoms.*

96 persons were assigned to the first group, and 35 to the second.

In order to follow the change in the C.S F by immunoelectrophoresis, in 77 (59 %) patients the C.S.F and the blood were re-examined 2—3 weeks after the onset of the disease. In the remaining 54 cases the patients had refused to cooperate or the *immunoelectrophoretic pattern had been normal* on the first occasion and repuncture therefore considered unnecessary an opinion that was later revised.

The proportion of convalescents was somewhat higher in the immunoelectrophoretically re-examined group than in the group that was not re-examined, namely 80 % against 69 % *Of the entire material, i.e* 131 patients, 91 % had

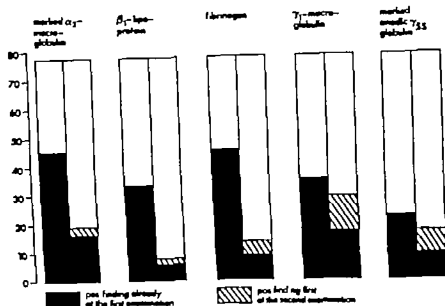


Diagram Number of precipitates in C.S.F from 77 patients.
Left column represents the number of precipitates at onset of disease.
Right column represents the number of precipitates 2—3 weeks after onset of disease.

and re-examination 2—3 weeks later and secondly the rate at which they appeared for the first time at the second examination. Each protein was compared separately with each of the remaining proteins studied. The rates with which the protein disappeared between the 2 examinations are given in Table 26.

The various proteins are given in the first column. The second gives the number of patients in whom the C.S.F showed precipitates on admission. The third and the fourth column give the number of patients who 2—3 weeks after the onset had one of the two proteins compared.

On comparison between the transproteins it was found that the β_1-lipoprotein and fibrinogen precipitates had disappeared more frequently than the marked α_2-macroglobulin precipitate. No difference was found between β_1-lipoprotein and fibrinogen precipitates. Comparison between immunoglobulins and transproteins, revealed a statistically significant larger decrease in the number of β_1-lipoprotein and fibrinogen precipitates than in that of the γ_1-macroglobulin precipitates. No substantial difference was found between γ_1-macroglobulin and marked α_2-macroglobulin precipitates. Of 27 persons with both proteins on admission, 1 had only marked α_2-macroglobulin precipitate after 2 weeks and 3 only

Table 24 Total protein content (mg per 100 ml.) in relation to severity of symptoms 2—3 weks after onset of disease

Total protein in mg. per 100 ml.	Convalescents		Still ill	
	N	per cent	N	per cent
≤50	32	84	6	16
51—100	19	61	12	39
>100	2	25	6	75

Table 25 Cell count (cells per cu.mm.) in relation to total protein content (mg. per 100 ml.) 2—3 weeks after onset of disease

Cells per cu. mm.	Total protein in mg. per 100 m		
	≤ 50	51—100	> 10
<5	14	5	
5—100	22	22	6
101—300	2	4	2

It is clear from Table 24 that in 38 patients or in half of all those examined the total protein content was normal. Most of the patients had practically recovered In 31 patients, of whom 19 were convalescent, it was between 51 and 100 mg per 100 ml. Of 8 patients with more than 100 mg per 100 ml. only 2 were convalescent. As in the beginning of the disease this variation of the frequency *of convalescents with the high total protein content was highly significant.*

In the beginning of the disease the cell count tended to be increased in patients with increased total protein content. The situation found at examination 2—3 weeks after onset of the disease is given in Table 25.

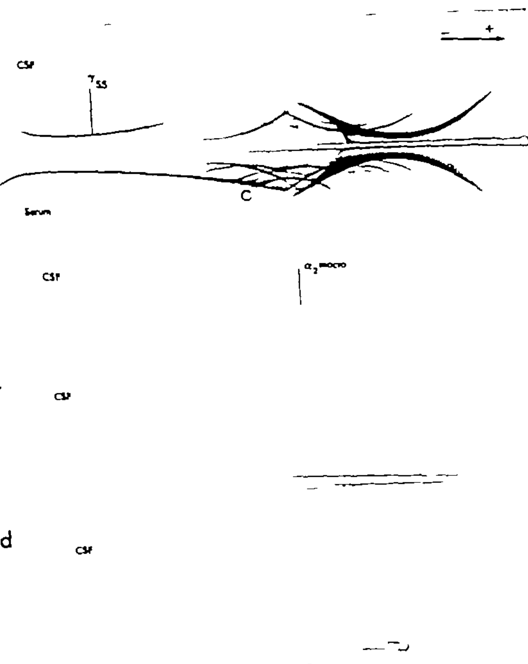
There was a distinct and almost statistically significant correlation between the severity of pleocytosis and the protein content.

IMMUNOELECTROPHORETIC STUDIES

2—3 *weeks after the onset of the disease, by which time most of the patients* were convalescent marked α_2-macroglobulin β_1-lipoprotein fibrinogen, γ_1-macroglobulin, and marked anodic γ_{ss}-globulin precipitates were much less common than at the onset of disease. Of 77 patients examined, the α_2-macroglobulin precipitate was marked in 19 (45) β_1-lipoprotein was seen in 8 (33) fibrinogen in 14 (45) γ_1-macroglobulin in 29 (35) and anodic γ_{ss}-globulin was marked in 17 (22) (The figures in brackets give the number of positive findings made in the same 77 persons on admission.) Concurrent immunoelectrophoresis of the serum in practically all of the cases never showed any abnormal precipitates. Diagram 1

The decreasing frequency of the findings did not demonstrably vary with sex or age. In order to assess the course, i.e. appearance and disappearance of the different proteins, the latter were studied in two different ways. Firstly regarding the rate at which the proteins disappeared between admission of the patients

-



…g Normal immunoelectrophoretic pattern in …S F and serum from case C T Feb 7 cf case …port Rabbit-antihuman-antiserum b Anti-α_2 macroglobulinserum. Sudan black staining and rabbit-antihuman-antiserum d Antifibrinogenserum.

Table 26 Comparison between incidence of immunoelectrophoretic findings made on admission and 2—3 weeks after onset of disease

		Both precip. at onset	First precip. at 2—3 weeks	Second precip. at 2—3 weeks
Marked α_2-macro	β lipo	24	6	
„	fibrinogen	31	8	
„	γ_1 macro	27	1	3
„	marked anodic γ_M	16	3	2
β lipo	fibrinogen	28	2	3
„	γ-macro	22		7
„	marked anodic γ_M	15		2
fibrinogen	γ-macro	28	1	10
	marked anodic γ_M	14	1	4
γ-macro	marked anodic γ_M	16	3	1

Table 27 Comparison between incidence of immunoelectrophoretic findings made on admission and 2—3 weeks after onset of disease

		No precip at onset	First precip. at 2—3 weeks	Second precip. at 2—3 weeks
Marked α_2-macro	β lipo	23		
„	fibrinogen	18		2
„	γ_1-macro	24	1	7
„	marked anodic γ_M	26	1	4
β lipo	fibrinogen	27		2
„	γ-macro	31		6
„	marked anodic γ_M	37	1	2
fibrinogen	γ macro	24		4
„	marked anodic γ_M	24	2	3
γ macro	marked anodic γ_M	36	7	

γ_1-macroglobulin at the second examination. As to the marked anodic γ_M-globulin precipitate, the number of patients with this precipitate was too small to warrant inclusion in such a comparison.

The opposite tendency *i.e.* the occurrence of the proteins found 2—3 weeks after admission but not on admission is given in Table 27

This table, which is constructed in the same way as Table 26 compares the various proteins with respect to the frequency with which they first appeared at the examination 2—3 weeks after onset of symptoms. Comparison between the 3 transproteins showed no significant difference. The table shows that the appearance of these proteins for the first time 2—3 weeks after onset of symptoms

was an exception. γ_1-macroglobulin occurred for the first time at the second examination more often than any of the transproteins. The same tendency though less marked, was noted for marked anodic γ_{2a}-globulin precipitate.

The following illustrative case report may serve as an example of the clinical picture and immunoelectrophoretic findings.

C.T. male, aged 7 years. Hitherto healthy.
Feb. 6. Acute headache, vomiting and fever (40° C)
Feb. 7 Admitted to hospital with pain and neck-stiffness. Otherwise normal. Temp. 38° C.

Examination on admission. Hb 12.3 gm. per 100 ml. RBC 4.4 mill. WBC 4,700. Diff. count. N 67 %, E nil, B nil, L 21 %, M 11 %. Atypical mononuclear cells 1 %. E.S.R. 12 mm/1 hr. Culture of faeces and throat swab for virus gave no growth. Complement fixation test for parotitis and adenovirus negative.

Feb. 13 Afebrile.
Feb. 7 Ophthalmoneurologically normal. EEG normal.
Feb. 20. Otoneurologically normal.
Feb. 2 Sent home symptomfree, when not exerting himself.
March 3 Re-examined and found to be healthy.

Cerebrospinal fluid findings

Date	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in CSF in paper electrophoresis	marked γ_1-macro-globulin	β_1-lipo-protein	fibri-nogen	γ_1-macro-globulin	marked anodic γ_2-globulin
7/2	18	98	normal					
20/2	15	59	normal				+	+

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS, SEX, AND AGE

It is clear from Table 28 that almost every fourth patient had a *marked* α_2-*macroglobulin* precipitate in the C.S.F. The frequency was somewhat lower (15 %) in the boys, but no difference was found between the remaining 3 groups. Only 8 persons showed β_1-*lipoprotein* at this examination. Only 1 of them was in the group below 15 years. The age distribution of the other patients was even. *Fibrinogen* was demonstrated in 14 patients at the examination 2—3 weeks after the onset of the symptoms. The proportion of patients with fibrinogen in 3 of the groups ranged from 13 to 15 % but in the remaining group, males above 15 years, it was somewhat higher namely 25 %. As many as 29 patients showed γ_1-*macroglobulin* in the C.S.F. It was thus the commonest finding at

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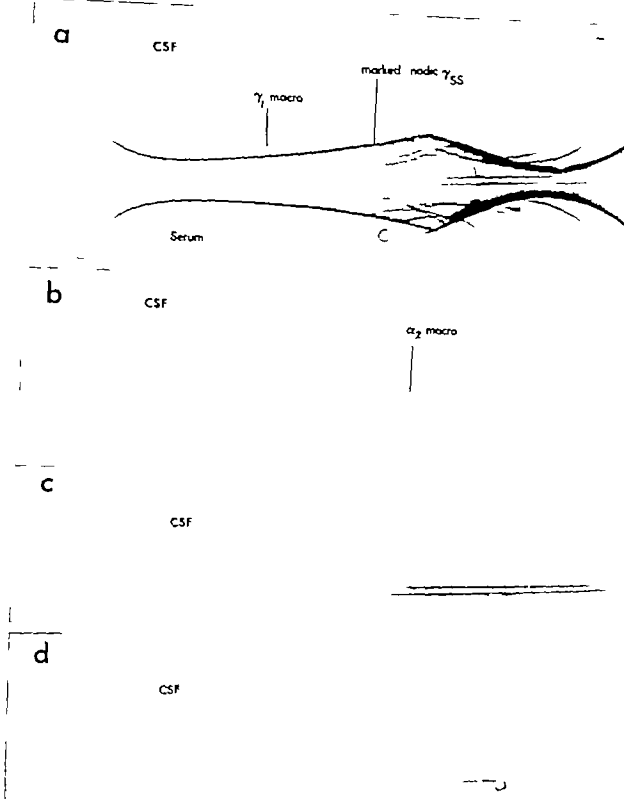


Fig. 11 Immunoelectrophoretic pattern in C.S.F. and serum from case O.T. Feb. 20 (cf. case report) a. Rabbit-antihuman-antiserum. b. Anti-α_2-macroglobulinserum. c. Sudan black staining and rabbit anti human-antiserum. d. Antifibrinogenserum. γ-macroglobulin and marked anodic γ_{ss}-globulin precipitate appear in C.S.F. at this phase of the disease.

γ_1-macroglobulin, *marked anodic γ_{ss}-globulin* precipitate was much less common (9 %) in the convalescents than in the group of still ill patients (50 %) The difference was highly significant.

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS CELL COUNT AND TOTAL PROTEIN CONTENT

In the beginning of the disease the frequency of precipitates varied with the severity of pleocytosis and certain proteins, Immunoglobulins and β_1-lipoprotein also tended to vary in frequency with the total protein content. The correlations found between these variables 2—3 weeks after the onset of symptoms are given in Tables 30—34.

Of the patients with a normal cell count, 16 % had a *marked α_2-macroglobulin* precipitate (Table 30) This protein was found in 26 % of those with 5—100 cells per cu.mm. and in 38 % of those with still more severe pleocytosis. The tendency of the protein to be more common in patients with a higher cell count was not statistically significant. On the other hand, only 21 % respecetively 13 % of those with a normal total protein content respectively 51—100 mg per 100 ml. had an increased α_2-macroglobulin precipitate against 88 % of those with a concentration above 100 mg per 100 ml. The difference was highly significant.

Of the patients with a normal cell count, only 5 % showed *β_1-lipoprotein* (Table 31) The protein was also seen in 8 % of those with a moderately increased cell count but in as many as 38 % of those who still had more than 100 cells per cu.mm. The tendency found was not statistically significant and was influenced by the fact that patients with more than 100 cells per cu.mm. also had a high total protein content. For the correlation between the total protein

Table 30 Marked α_2-macroglobulin precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 00 ml.) —3 weeks after onset of disease

Total protein in mg per 00 ml.	≤ 50		5 — 00		> 00		
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	4		5				16
5— 00	[illegible]	6	[illegible]		6	5	26
—300			4			[illegible]	38
Per cent				3		88	

Table 28 Classification of patients according to sex, age, and immunoelectrophoretic findings 2—3 weeks after onset of disease

Sex and age		N	marked α_2-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_m-globulin	
			N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
Males	<15	13	2	15			2	15	2	15	1	8
"	>15	35	10	29	4	11	8	23	14	40	10	29
Females	<15	8	2	25	1	13	1	13	2	25	1	13
"	>15	21	5	24	3	14	3	14	11	52	5	24

Table 29 Correlation between immunoelectrophoretic findings and severity of symptoms 2—3 weeks after onset of disease

Clinical symptoms	N	marked globulin		macro- β_1-lipoprotein		fibrinogen		γ_1-macro-globulin		marked anodic γ_m-globulin	
		N	per cent	N	per cent	N	per cent	N	per cent	N	per cent
Convalescents	53	10	19			8	15	14	26	5	9
Still ill	24	9	38	8	33	6	25	15	63	12	50

this examination 2 3 weeks after onset of symptoms. Only 4 (19 %) patients were below 15 years but as many as 45 % of those above 15 years, showed γ_1-macroglobulin The frequency of this protein did not vary with sex. *Marked anodic γ_m-globulin* precipitate was found in 17 patients. Only 2 (10 %) were below 15 years against about 27 % of those above this age limit.

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS AND SEVERITY OF SYMPTOMS

As previously mentioned 53 patients were convalescent while 24 were still ill. *Marked α_2-macroglobulin* precipitate was found in 19 % of the convalescents and in 38 % of those who were still ill but the difference was not statistically significant. In none of the convalescents was *β_1-lipoprotein* found 2 weeks after the onset of the disease. All 8 thus belonged to the group who were still ill and the difference, 0 % against 33 % was highly significant. *Fibrinogen* was demonstrated in 15 % of the convalescents against 25 % in those who were still ill, but the difference was *not* significant. As many as 63 % of the 24 patients who were still ill showed *γ-macroglobulin* against only 26 % of those who were convalescent. The difference was statistically significant. Like β lipoprotein and

Table 34 Marked anodic γ_m-globulin precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 100 ml.) 2—3 weeks after onset of disease

Total protein in mg. per 100 ml.	≤ 50		51 — 100		> 100		
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.	per cent
<5	4		5	2			
5—100	22		22	8	6		24
—500	2		4		2		38
Per cent		5		25		50	

content and the frequency of β_1-lipoprotein was significant. None of the patients with a normal total protein content showed this protein, and only 10 % of those with up to 100 mg. per 100 ml. against as many as 63 % of those with a still higher total protein content.

It is clear from Table 32 that no correlation was found between the frequency of *fibrinogen* precipitate and the cell count. On the other hand, a highly significant correlation was found between the frequency of the precipitate and the total protein content. Only 8 % and 16 % of those who had normal respectively moderately increased total protein content showed fibrinogen, against as many as 75 % of those who still had more than 100 mg. per 100 ml.

Of the patients with a normal cell count γ_1-*macroglobulin* was found in 21 % (Table 33). It was demonstrated in 42 % and 50 % of those with a cell count up to 100 per cu.mm. respectively 500 per cu.mm. This correlation with the severity of pleocytosis was, however, not so close as that with the total protein content, only 16 % of those with a normal total protein content showed this protein, but as many as 55 % and 75 % of those in the groups with a still higher concentration of the total protein. The correlation was highly significant.

The frequency of *marked anodic* γ_m-*globulin* precipitate, which was shown in 15 cases, tended to vary with the severity of pleocytosis (Table 34) but this tendency was due in part to the relatively high frequency of increased total protein content in those with more than 5 cells per cu.mm. For the correlation between the frequency of marked γ_m-globulin precipitate and the total protein content was highly significant. Only 5 % of those with a normal total protein content showed marked γ_m-globulin precipitate, against as many as 35 % respectively 50 % of those with still larger total protein contents.

Table 31 β-lipoprotein precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 100 ml.) 2—3 weeks after onset of disease

Total protein in mg per 100 ml	≤ 50		51—100		> 100		
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.	per cent
< 5	14		5	1			*5*
5—100	22		22	1	6	3	*8*
101—500	2		4	*1*	2	2	*38*
Per cent				*10*		*63*	

Table 32 Fibrinogen precipitates in relation to cell content (cells per cu. mm.) and total protein content (mg. per 100 ml.) 2—3 weeks after onset of disease

Total protein in mg per 100 ml	≤ 50		51—100		> 100		
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.	per cent
< 5	14	1	5	3			*21*
5—100	22	2	22	2	6	4	*16*
101—500	2		4		2	2	*25*
Per cent		*8*		*16*		*75*	

Table 33 γ-macroglobulin precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 100 ml.) 2—3 weeks after onset of disease

Total protein in mg per 100 ml.	≤ 50		51—100		> 100		
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.	per cent
< 5	14	2	5	2			*21*
5—100	22	4	2	12	6	5	*42*
101—500	2		4	3	2	1	*50*
Per cent		*16*		*55*		*75*	

patients was the immunoelectrophoretic pattern normal. Of the 2 patients infected with enterovirus, the one with poliomyelitis had a total protein content of 171 mg per 100 ml. and she showed β_1-lipoprotein, fibrinogen, γ-macroglobulin and marked anodic γ_m-globulin precipitates. The other patient infected with Coxsackie A 8 with a total protein content of 61 mg. per 100 ml. showed a normal protein pattern. Of the 2 patients with adenovirus meningoencephalitis and a total protein concentration of 49 respectively 58 mg per 100 ml. one showed γ_1-macroglobulin and the other marked anodic γ_m-globulin precipitate. The two patients with influenza virus meningoencephalitis had 58 respectively 54 mg per 100 ml. total protein. In one the immunoelectrophoretic pattern was normal, while the other showed γ_1-macro- and marked anodic γ_m-globulin precipitates. One patient with herpes zoster meningoencephalitis and 2 with measles meningoencephalitis showed a normal immunoelectrophoretic pattern. The total protein concentration was 58 respectively 36 and 40 mg. per 100 ml.

DISCUSSION

As mentioned previously in 77 patients the sera and C.S.F were studied 2—3 weeks after onset of the disease. Since the main reason why 54 patients were not re-examined was that they had recovered, there was reason to expect that the true proportion of patients still ill in the re-examined group was larger than in the group not re-examined. The difference was, however not so very large, namely 80 % against 69 %

As a rule, the total protein content and the cell count tended to become normal during the first 2—3 weeks after the onset of the symptoms. The cell count was normal in 19, and the total protein content in half of all the patients examined.

The frequency of pathologic precipitates in the C.S.F in the 77 patients was lower at the re-examination. γ_1-macroglobulin, which was the commonest finding at this control, was found in 29 persons, marked α_2-macroglobulin precipitate in 19, marked anodic γ-globulin precipitate in 17 fibrinogen in 14 and β_1-lipoprotein in 8. On admission, on the other hand, marked α_2-macroglobulin precipitate and fibrinogen had been the commonest findings. Of the 14 fibrinogen precipitates there was reason to suspect degradation products of fibrinogen as the responsible antigen in only 5.

The frequency of immunoglobulin precipitates varied considerably with age. Thus, of the 21 patients below 15 years only 2 showed marked anodic γ_m-globulin

Table 35 Immunoelectrophoretic findings in patients grouped according to type of meningoencephalitis 2—3 weeks after onset of disease

Diagnosis	N	marked α_1-macroglobulin		β_1-lipoprotein		fibrinogen		γ_1-macroglobulin		marked anodic γ_m-globulin	
		N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.	N pos.	per cent pos.
Mumps	17	3	18			2	11	3	18	3	18
Miscellaneous	60	16	26	8	13	12	20	26	43	14	23

Table 36 Immunoelectrophoretic findings in 13 patients with known type of meningoencephalitis 2—3 weeks after onset of disease

Diagnosis	N	marked α_1-macroglobulin	β_1-lipoprotein	fibrinogen	γ macroglobulin	marked anodic γ_m globulin
RSSE	4	2	1	3	4	2
Enterovirus	2		1	1	1	1
Adenovirus	2				1	1
Influenza	2				1	1
Herpes zoster	1					
Measles	2					

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS AND VIROLOGIC DIAGNOSIS

Of the 77 patients studied, 17 had mumps meningoencephalitis. Of the remaining 60 the virus had been determined in 13

Of the 17 patients with mumps meningoencephalitis the immunoelectrophoretic pattern was normal in 11 which means that the findings given in Table 35 were made in 6 patients. Of the 60 patients with some other type of meningoencephalitis the immunoelectrophoretic pattern was normal in 27 and abnormal in 33 The two groups are too small to warrant statistical comparison but γ_1-macroglobulin precipitate was less common in the mumps meningoencephalitis group.

The distribution of precipitates among the various types of meningoencephalitis is given in Table 36

In the 4 patients with RSSE, in whom the total protein content ranged between 69 and 291 mg per 100 ml. (average 125 mg per 100 ml.) the number of abnormal findings was larger than in any of the other groups. In none of the 4

almost the same as on admission, while the frequency of transproteins was much lower On comparison of the findings made in each individual on admission with those made at the re-examination, however it was found that of the transproteins, β_1-lipoprotein and fibrinogen had disappeared more often than a marked α_2-macroglobulin precipitate. No difference was found between marked α_2-macroglobulin and γ_1-macroglobulin in this respect. But on inverse comparison, differences were found between these 2 Of 24 patients who showed no macroglobulins on admission, 7 showed γ_1-macroglobulin in the C.S.F after 2 weeks, but only 1 a marked α_2-macroglobulin precipitate. Thus γ_1-macroglobulin made its initial appearance in the C.S.F 2—3 weeks after onset more often than the 3 transproteins. This appearance of γ_1-macroglobulin had occurred despite clinical improvement of the patients. The total protein content decreased in all 7 but had reached normal in only 1

The number of patients with a marked anodic γ_m-globulin precipitate was not large enough to allow reliable comparison. Suffice it therefore to state that of 22 persons who showed a marked anodic γ_m-globulin precipitate on admission, 15 did not show this precipitate 2—3 weeks later and 8 persons who had not a marked anodic γ_m-globulin precipitate on admission showed this protein after 2 weeks.

It was noteworthy that as many as 29 persons showed γ_1-macroglobulin in the C.S.F 2—3 weeks after the onset of the disease. 17 of them had also shown γ_1-macroglobulin on admission, but in 12 this precipitate had occurred later It is astonishing that the γ_1-macroglobulin was found as the only large molecular precipitate in 9 cases and together with a marked anodic γ_m-globulin precipitate in a further 6. In 15 patients then the only immunoelectrophoretic abnormalities of the protein pattern were increased amounts of these 2 immunoglobulins, though 6 of them had a normal total protein content. The increased content of immunoglobulins was probably due to the prolonged antibody formation in viral diseases. For comparison, it might be mentioned that of 17 patients with bacterial meningitis, 9 showed γ_1-macroglobulin in the C.S.F on admission. After adequate treatment only 1 patient had γ_1-macroglobulin in the C.S.F after 2 weeks. In order to form an opinion as to whether the selective increase of immunoglobulins in the C.S.F is accompanied by an increased serum concentration, the serum was also studied with paper and immunoelectrophoresis. The paper electrophoresis showed normal γ-globulin-fractions in 24 of the subjects. In 4 the γ-globulin concentration was slightly increased. The highest value recorded was 1 51 mg per 100 ml. and in 1 case the absolute value is not known. In 70 cases the serum concentration of γ_1-macroglobulin were roughly estimated from

and 4 γ_1-macroglobulin precipitate, against 15 respectively 25 out of 56 above 15 years. The groups were, however, not large enough to allow any conclusion about the statistical significance of this difference. *Even on admission*, a marked anodic γ_m-globulin precipitate was less common in patients below than in those above, 15 years.

The immunoelectrophoretic pattern was normal in 11 of 17 patients with mumps meningoencephalitis and in 20 of 60 with other types of meningoencephalitis. The frequency of γ macroglobulin precipitates was lower in patients with mumps meningoencephalitis. Those with RSSE showed relatively many precipitates. All 4 showed γ macroglobulin in the C.S.F and 2 of them also marked anodic γ_m-globulin precipitate.

In the first phase of *meningoencephalitis a correlation was found* between the increase of the total protein content and the presence of large plasma proteins in the C.S.F which was ascribed to an "open barrier" This correlation was still stronger at the examination 2 weeks after the onset of the symptoms, and then for all 5 proteins studied. Simple division of the patients into 2 groups, one with a normal and one with an increased total protein content, which has proved useful in clinical practice, did not prove a satisfactory measure of the extent of injury to the blood-C.S.F barrier as is apparent from the following comparison. On admission 43 persons had a normal total protein content and 23 % of them had no large molecular proteins in the C.S.F At 2—3 weeks after onset of symptoms 38 persons had a normal total protein concentration *and 70 % of them showed a normal immunoelectrophoretic protein pattern* of the C.S.F In some cases the blood-C.S.F barrier had thus recovered its capacity to prevent the passage of large protein molecules, which was not apparent from measurement of the total protein content.

A highly significant correlation was found between the clinical symptoms and the total protein content. As therefore expected, the pathological precipitates were less common in persons who had recovered most. The correlation with β_1-lipoprotein and the immunoglobulins was highly significant. But, as on admission, the correlations between the severity of the symptoms and fibrinogen respectively α_2-macroglobulin were not so close.

The individual patients were also studied for differences in the duration and time of initial appearance of the pathological precipitates in the C.S.F For this purpose each protein was compared separately with each of the other proteins in each patient. The results obtained differed from those obtained on simple comparison of the number of precipitates. For the latter comparison showed that 2—3 weeks after the onset *of the symptoms* the number of immunoglobulins was

γ_1-macroglobulin and a marked anodic γ_s-globulin precipitate were more common than any of the other proteins in patients who had no such precipitates on admission. The findings argue for the assumption of a decreased passage of serum proteins into the C.S.F. and lend strong support to the hypothesis that immunoglobulins are formed in the intrathecal space.

Examination 6—10 Weeks after Onset of Disease

MATERIAL

Of the 131 patients, 101 were re-examined 6—10 weeks after the onset of symptoms. Of the remaining 30 patients, 15 had recovered before this re-examination and 15 refused to cooperate.

Of the 101 patients, 74 had recovered by the time of the re-examination and were discharged as healthy. This brought the total number of recoveries up to 89 (77 % of 116). Here recovery is to be understood as freedom from symptoms apart from weakness not severe enough to prevent the patients from returning to school or work.

The C.S.F. was examined at the onset of the disease in 131 cases, again 2—3 weeks after the onset in 77 and 6—10 weeks after the onset in 36. In those cases in which the C.S.F. was not re-examined it was mostly because the immunoelectrophoretic pattern had proved normal at the previous examination or because the patient had in the meantime recovered and refused lumbar puncture.

Of these 36 in whom the C.S.F. was re-examined, 16 were still ill. The symptoms were mainly fatigue and headache. Two patients were subfebrile, 1 was receiving treatment in a respirator and 3 had psychic symptoms. Four of them required hospital care. These 36 patients are grouped according to sex and age and the clinical picture in Table 37, which shows that the recovery rate did not vary with sex and that it was possibly somewhat higher in the younger group.

THE CELL COUNT

The number of cells in the C.S.F. had progressively decreased, and in most of the patients the count was at most only slightly increased. When classified in the same way as previously the uneven distribution in the group 5—100 cells was

the position and the appearance of the precipitate in the immunoelectrophoretic pattern. In serum from 28 with γ_1-macroglobulin in the C.S.F. 8 were found to be +++, 7 ++ and 11 +. Two of the sera did not show this precipitate. Of 42 without γ_1-macroglobulin in the CSF 8 were +++, 19 ++, 14 +. One person showed no γ_1-macroglobulin in the serum. There was thus no substantial difference between the 2 groups. On comparison between the serum precipitates in the 11 in whom γ_1-macroglobulins appeared in the C.S.F. for the first time after 2 weeks the γ_1-macroglobulin precipitate in the serum increased markedly in only 2. This comparison and the fact that the blood-C.S.F. barrier had in the meantime recovered for the transproteins α_2-macroglobulin β_1-lipoprotein and fibrinogen could usually no longer be demonstrated, lend strong support to the hypothesis that the immunoglobulins had been produced in the intrathecal space.

SUMMARY

131 persons were examined 2—3 weeks after onset of meningoencephalitis. On clinical grounds the cases were divided into 2 groups: 96 were classified as convalescents and 35 as still ill.

C.S.F. from 77 persons and sera from 70 were analysed. The cell count was normal in 19 and the total protein content in 38. A highly significant correlation was found between the severity of the disease and the total protein content. The correlation between the severity of the disease and the cell count was not statistically significant. A marked α_2-macroglobulin precipitate was found in 19, β_1-lipoprotein in 8, fibrinogen in 14, γ_1-macroglobulin in 29 and marked anodic γ_{2a}-globulin precipitate in 17. γ_1-macroglobulin was thus the commonest finding and was found in 6 patients with a normal total protein content.

The immunoglobulin precipitate was found more often in patients above 15 years. β_1-lipoprotein, γ_1-macro- and marked anodic γ_{2a}-globulin precipitates were statistically more common among those who were still ill.

No statistically significant correlation was found between cell count and the proteins demonstrated. On the other hand, a highly significant correlation was found between the total protein concentration and the number of precipitates demonstrated. In 29 % of the patients with a normal total protein concentration the immunoelectrophoretic pattern was, however, not normal.

Between admission and 2—3 weeks after onset of disease it was found that the β_1-lipoprotein and fibrinogen had disappeared to a much larger extent than a marked α_2-macroglobulin precipitate and γ_1-macroglobulin. On the other hand,

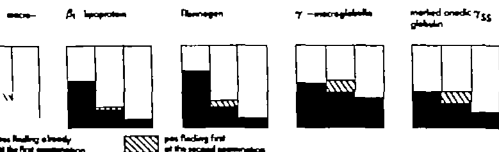


Diagram 2. Number of precipitates in C.S.F from 8 patients on repeated examinations. Left column represents the number of precipitates at onset of disease. Middle column represents the number of precipitates 2—3 weeks after onset of disease. Right column represents the number of precipitates 6— weeks after onset of disease.

IMMUNOELECTROPHORETIC STUDIES

The C.S.F and serum were studied immunoelectrophoretically in 36 cases, including 8 in which the C.S.F was not studied 2—3 weeks after the onset of symptoms. Therefore only the 28 patients who took part in both re-examinations were included in the study of the variation in the number of precipitates from one occasion to another The figures in brackets give the number of precipitates seen 2—3 weeks after onset. The numbers of precipitates recorded were as follows marked α_2-macroglobulin 3 (12) β_1-lipoprotein 3 (7) fibrinogen 3 (9) γ_1-macroglobulin 10 (16) marked anodic γ_{ss}-globulin 5 (12) The number of precipitates had thus decreased considerably The commonest finding was γ_1-macroglobulin, as at the re-examination 2—3 weeks after the onset of symptoms. All precipitates were demonstrated in 15 persons, while the immunoelectrophoretic pattern in the remaining 21 was normal.

As in the previous chapter each component was compared separately with each of the other components.

No differences were found between the 3 transproteins. They all tended to return to normal at roughly the same rate. On the other hand, a difference was found between the 3 transproteins, on one hand, and the heavy immunoglobulin on the other (Table 40) γ_1-macroglobulin tended to return to normal somewhat slower Marked anodic γ_{ss}-globulin precipitate disappeared from the C.S.F at the same rate as the transproteins. No cases showed the opposite tendency i.e. precipitates for the first time 6—10 weeks after onset of the symptoms.

Table 37 Classification of patients according to sex, age and condition 6—10 weeks after onset of disease

Sex and age *in years*		Recovered		Still ill	
		N	per cent	N	per cent
Males	<15	1	100		
„	>15	12	55	10	45
Females	<15	3	75	1	25
„	>15	4	44	5	56

Table 38 Classification of patients according to cell content of C.S.F. (cells per cu.mm.) and condition 6—10 weeks after onset of disease

Cells per cu. mm.	Recovered		Still ill	
	N	per cent	N	per cent
<5	14	78	4	22
5—10	6	55	5	45
11—50			4	100
>50			3	100

Table 39 Total protein content (mg. per 100 ml.) in relation to condition 6—10 weeks after onset of disease

Total protein in mg. per 100 ml.	Recovered		Still ill	
	N	per cent	N	per cent
≤50	15	68	7	32
51—100	5	45	6	55
>100			3	100

not seen for which reason different class intervals were used in the classification of the cells.

At this re-examination half of the patients had a normal number of cells in the C.S.F. and of these, 78 % had recovered. Of the other half with slight pleocytosis, (only 7 had more than 10 cells per cu.mm.) only 39 % had recovered. Pleocytosis was thus more common in the patients who were still ill.

TOTAL PROTEIN CONTENT

The total protein content also tended to return to normal (Table 39).

Among those with a normal total protein content the number of recoveries (68 %) was thus higher than the number who were still ill (32 %). Of the 14 patients in whom the total protein content was still increased, 5 (36 %) had recovered and 9 were still ill. The variation within the group with 51—100 mg per 100 ml. did not differ so much in the two clinical groups as to justify subgrouping. The mean found for the 5 who had recovered was 56 mg. per 100 ml., and for the patients still ill it was 64 mg. per 100 ml.

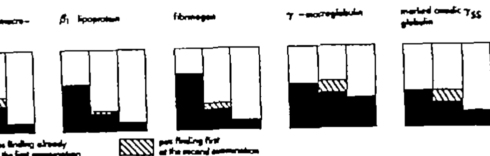


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IMMUNOELECTROPHORETIC STUDIES

The C.S.F and serum were studied immunoelectrophoretically in 36 cases, including 8 in which the C.S.F was not studied 2—3 weeks after the onset of symptoms. Therefore only the 28 patients who took part in both re-examinations were included in the study of the variation in the number of precipitates from one occasion to another The figures in brackets give the number of precipitates seen 2—3 weeks after onset. The numbers of precipitates recorded were as follows marked α_1-macroglobulin 3 (12) β_1-lipoprotein 3 (7) fibrinogen 3 (9) γ_1-macroglobulin 10 (16) marked anodic γ_{ss}-globulin 5 (12) The number of precipitates had thus decreased considerably The commonest finding was γ_1-macroglobulin, as at the re-examination 2—3 weeks after the onset of symptoms. All precipitates were demonstrated in 15 persons, while the immunoelectrophoretic pattern in the remaining 21 was normal.

As in the previous chapter each component was compared separately with each of the other components.

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Table 40 Comparison between incidence of immunoelectrophoretic findings at 2—3 weeks and 6—10 weeks after onset of disease

	Both precip. at 2—3 weeks	First precip. at 6—10 weeks	Second precip. at 6—10 weeks
Marked α_1-macro β lipo	7	1	2
" fibrinogen	7	1	2
" γ-macro	10		3
" marked anodic γ_m	7	1	2
β lipo fibrinogen	5	1	1
" γ macro	6		3
" marked anodic γ_m	6		1
fibrinogen γ macro	7		2
" marked anodic γ_m	5	1	1
γ-macro marked anodic γ_m	9	4	

The following illustrative case report may serve as an example of the clinical picture and immunoelectrophoretic findings.

F.S., a male, aged 55 years. Hitherto healthy
August 23 Headache, vomiting, myalgia and fever (39 C)
August 30 Admitted to hospital because of persistent symptoms. Patient ill with lowered level of consciousness. Neck-stiffness. Fever (39.5 C)

Examination on admission Hb. 13.2 gm. per 100 ml. RBC 4.2 mill. WBC 4,400. Diff count. N 78 %, E 1.5 %, B 0.5 %, L 17 % M 2 % E.S.R. 32 mm./1 hr
Culture of C.S.F for bacteria gave no growth. Culture of faeces for virus negative. Complement fixation test for parotitis, influenza and ornithosis negative. Bunnell's test, cold agglutination test and antistreptolysin titre negative.

Sept 8 E.E.G showed theta activity with left sided dominance.
Sept 9. Nystagmography showed abnormality as in encephalitis.
Sept 12 Ophthalmologically normal. Afebrile.
Sept 14. Patient sent home.
Oct 31 Patient re-examined and found not healthy

Cerebrospinal fluid findings

Date	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in CSF in paper electrophoresis	marked α_1-macro-globulin	β_1-lipo-protein	fibrinogen	γ_1-macro-globulin	marked anodic γ_m-globulin
30/8	71.8	73	normal				+	+
13/9	19.3	58	normal				+	+
31/10	12	42	normal					

Table 4 Classification of patients according to sex, age and immunoelectrophoretic findings 6—1 weeks after onset of disease

Sex and age in years		N	Marked α_2-macroglobulin	β_1-lipoprotein	Fibrinogen	γ_1-macroglobulin	marked anodic γ_2-globulin
			N pos.	N pos.	N pos.	N pos.	N pos.
Males	< 5						
	> 5	11			1	5	4
Females	< 5	4					
	> 5	9				3	1

Table 4a Marked α_2-macroglobulin precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 00 ml.) 6— weeks after onset of disease

Total protein mg. per 00 ml.	≤ 50		5 —100		> 00	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N. pos.
<5	3		5			
5—1	6		4			
—50	3					
>50						

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS SEX, AND AGE

Only 5 of the patients re-examined were below 15 years. Of the remaining 31 most were males. Marked anodic γ_2-globulin precipitates were seen in none of the 5 children while γ-macroglobulin was noted in 2 Of the 31 patients above 15 years, marked anodic γ_2-globulin precipitate was found in 5 and γ_1-macroglobulin in 8.

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS AND CONDITION CELL COUNT AND TOTAL PROTEIN CONTENT

Marked α_2-macroglobulin precipitate was demonstrated in the C.S.F in 2 subjects in whom the total protein content was normal. Both patients, who also showed γ_1-macroglobulin, were still ill. In the third patient, who had not recovered either the total protein content was 244 mg. per 100 ml. and showed all 5 pathologic proteins.

Table 43 β lipoprotein precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 100 ml.) 6—10 weeks after onset of disease

Total protein mg. per 100 ml.	≤ 50		51—100		> 100	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	13		5			
5—10	6		4		1	1
11—50	3		1			
>50			1		2	2

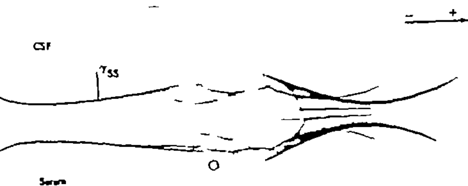
Table 44 Fibrinogen precipitate in relation to cell count *(cells per cu.mm.) and total protein content (mg. per* 100 ml.) 6—10 weeks after onset of disease

Total protein mg per 100 ml.	≤ 50		51—100		> 100	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	13	1	5			
5—10	6	2	4		1	1
11—50	3		1			
>50			1		2	1

It is clear from the table that only 3 *β_1-lipoprotein* precipitates were demonstrated in this stage of the disease. All 3 patients were still ill and in all the total protein content in the C.S.F. was high. β_1-lipoprotein was not demonstrable in any of these cases as the only finding

Five patients showed *fibrinogen* precipitates. In 2 both females who had recovered, fibrinogen was the only precipitate found. Both had previously been punctured on admission only and then fibrinogen had also been found in the C.S.F. At the re-examination the total protein content was normal in both, and in one of them the *number of cells* was slightly increased In the 3 remaining patients, who were still ill the C.S.F. showed several other proteins which normally do not occur in the C.S.F. In 2 of them the total protein content was markedly increased

γ_1-macroglobulin precipitate was demonstrated in 7 of 10 patients with a normal total protein content. Of 18 patients with a normal number of cells, only 2



CSF

α_2 macro

CSF

d CSF

Normal immunoelectrophoretic pattern in and serum from case F.S. Aug 3 of case Rabbit-anti human-antiserum b Anti-α_2 macroglobulinserum. c. Sudan black staining and rabbit-antihuman-antiserum d Antifibrinogenserum.

Table 43 β lipoprotein precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 100 ml.) 6—10 weeks after onset of disease

Total protein mg. per 100 ml.	≤ 50		51—100		> 100	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	13		5			
5—10	6		4		1	1
11—50	3		1			
>50			1		2	2

Table 44 Fibrinogen precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg per 100 ml.) 6—10 weeks after onset of disease

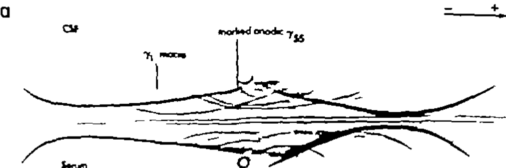
Total protein mg per 100 ml.	≤ 50		51—100		> 100	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	13	1	5			
5—10	6	2	4		1	1
11—50	3		1			
>50			1		2	1

It is clear from the table that only 3 β_1-*lipoprotein* precipitates were demonstrated in this stage of the disease. All 3 patients were still ill and in all the total protein content in the C.S.F was high. β_1-lipoprotein was not demonstrable in any of these cases as the only finding

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γ_1-*macroglobulin* precipitate was demonstrated in 7 of 10 patients with a normal total protein content. Of 18 patients with a normal number of cells, only 2

4. Immunoelectrophoretic pattern in C.S.F. and m from case F.S. Oct. 3 (cf. case report) -bbit-antihuman-antiserum. b. Anti-α_2-macro-bulinserum. c. Sudan black staining and rabbit antihuman-antiserum. d. Antifibrinogenserum. γ-macroglobulin and marked anodic γ_{ss}-globulin still appear









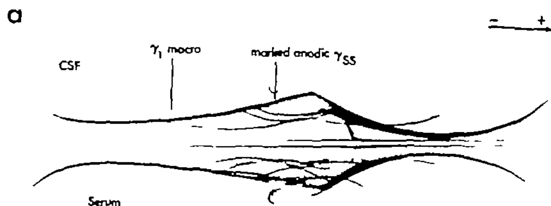








Fig 5 Immunoelectrophoret pattern C S F and serum from case F.S Sep. 3 (cf case report) a. Rabbit antihuman-antiserum. b. Anti-α_2 macroglobulinserum. c. Sudan black staining and rabbit antihuman-antiserum. d. Antifibrinogenserum. -macroglobulin nd ma ked anodic γ_{ss}-globuli precipitates ppear in C.S.F at this phase of th disease

Table 45 γ-macroglobulin precipitate in relation to cell count (cell per cu.mm.) and total protein content (mg. per 100 ml.) 6— weeks after onset of disease

Total protein mg. per 100 ml.	≤ 50		5 —100		> 90	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	13	2	5			
5—	6	3	4		1	1
—50	3	2	1			
>50						

Table 46 Marked anodic γ_m-globulin precipitate in relation to cell count (cells per cu.mm.) and total protein content (mg. per 00 ml.) 6—1 weeks after onset of disease

Total protein mg. per 100 ml.	≤ 50		5 —100		> 90	
Cells per cu. mm.	N	N pos.	N	N pos.	N	N pos.
<5	3		5			
5—1	6		4			
—50	3		1			
>50						

showed γ_1-macroglobulin in increased concentration, against 8 of an equally large number of patients with pleocytosis. Four patients, including 3 who had recovered, showed γ_1-macroglobulin as the only immunoelectrophoretic abnormality. One male who had not recovered showed a normal total protein content and both immunoglobulin precipitates, but no transprotein precipitates. In 5 who were still ill this protein was found together with a marked α_2-macroglobulin precipitate in 2 and in 1 person with fibrinogen. Two of the patients who had not recovered showed, 6—10 weeks after the onset of the disease, not only γ_1-macroglobulin but also several other pathological precipitates.

A *marked anodic* γ_m-*globulin* precipitate was the only pathological finding in 2 patients who had not recovered. The total protein content in these 2 was slightly increased and one had 29 cells per cu.mm. One patient, who was still ill and in whom the total protein concentration was normal, showed γ_1-macro-

5 — Ursing



γ_1-macroglobulin as the only pathological precipitate. Another patient showed not only γ_1-macroglobulin but also fibrinogen and β_1-lipoprotein. In 2 of these patients the immunoelectrophoretic pattern was normal.

One patient with adenovirus infection had a total protein content of 55 mg per 100 ml. and showed a marked anodic γ_{ss}-globulin precipitate. In the patient with influenza virus meningoencephalitis the total protein content was 42 mg. per 100 ml. and the immunoelectrophoretic pattern showed γ_1-macroglobulin. The girl with German measles meningoencephalitis had 48 mg per 100 ml. and showed a normal immunoelectrophoretic pattern.

DISCUSSION

The 36 patients in whom the C.S.F and serum were reexamined 6—10 weeks after the onset of symptoms were not really representative of the entire material because in most of the patients who had recovered the C.S.F was not analysed. Of 116 persons re-examined 6—10 weeks after the onset of the symptoms, 77 % had recovered, against only 56 % of those in whom the C.S.F had been analysed.

In most, but not all, of the 16 who were still ill, the cell count was higher and the total protein content larger than in those who had recovered. As a rule, not only the total protein content and the cell count, but also the clinical picture as well as the immunoelectrophoretic protein pattern improved simultaneously On this occasion a marked α_2-macroglobulin precipitate was found in 5 cases, β_1-lipoprotein in 3, fibrinogen in 5, γ_1-macro- in 10 and a marked anodic γ_{ss}-globulin precipitate in 5. Of 20 patients who had recovered, only 5 still showed pathologic precipitates in the C.S.F., but of 16 who were still ill, 10 showed a pathological protein pattern at re-examination 6—10 weeks after the onset of symptoms. Among these 15 in whom the immunoelectrophoretic protein pattern was not normal, only 1 belonged to the mumps meningoencephalitis grup. In the other 7 with this diagnosis the pattern had become normal.

Of 28 immunoglobulin precipitates observed at the previous examination, 15 persisted, against only 9 of 28 transprotein precipitates, which shows that it was above all the α_2-macroglobulin, β_1-lipoprotein and fibrinogen that decreased in concentration with the patient's recovery On comparison of each precipitate with each of the other precipitates in each patient, it was also found that the γ_1-macroglobulin precipitate often persisted longer than the 3 transproteins and

Table 47 Immunoelectrophoretic findings in patients grouped according to type of meningoencephalitis 6—10 weeks after onset of disease

Diagnosis	*N*	marked α_2-macro-globulin	β-lipo-protein	fibri-nogen	γ macro-globulin	marked anodic γ_m-globulin
Mumps	8			1		
Miscellaneous	28	3		4	10	5

Table 48 Immunoelectrophoretic findings in 7 patients with known type of meningoencephalitis 6—10 weeks after onset of disease

Diagnosis	*N*	marked α_2-macro-globulin	β-lipo-protein	fibri-nogen	γ-macro-globulin	marked anodic γ_m-globulin
RSSE	4		1	1	2	
Adeno virus	1					1
Influenza	1				1	
Rubeola	1					

globulin and marked anodic γ_m-globulin precipitate. Two patients, who were still ill and in whom the total protein content was increased, showed marked anodic γ_m-globulin precipitates together with several other proteins not normally occurring in the C.S.F

CORRELATION BETWEEN IMMUNOELECTROPHORETIC FINDINGS AND *VIROLOGIC DIAGNOSIS*

Of 36 patients re-examined 6—10 weeks after onset of disease, 8 had mumps meningoencephalitis. Of the remaining 28 a specific virological diagnosis had been made in 7

Of the 8 patients who had had mumps meningoencephalitis, all showed a normal immunoelectrophoretic pattern except 1 in whom a fibrinogen precipitate was noted. All cases with immunoglobulin precipitates were thus found *in the patients* who had had other forms of meningoencephalitis.

The precipitates found at re-examination 6—10 weeks *after* the onset of symptoms in 7 patients in whom the virus had been determined are given in Table 48

Four patients with RSSE were *re-examined*. The total protein content ranged from 35 to 120 mg per 100 ml. (Average 72 7 mg per 100 ml.) One showed

γ_1-macroglobulin as the only pathological precipitate. Another patient showed not only γ_1-macroglobulin but also fibrinogen and β_1-lipoprotein. In 2 of these patients the immunoelectrophoretic pattern was normal.

One patient with adenovirus infection had a total protein content of 53 mg per 100 ml. and showed a marked anodic γ_{ss}-globulin precipitate. In the patient with influenza virus meningoencephalitis the total protein content was 42 mg. per 100 ml. and the immunoelectrophoretic pattern showed γ_1-macroglobulin. The girl with German measles meningoencephalitis had 48 mg. per 100 ml. and showed a normal immunoelectrophoretic pattern.

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In most, but not all, of the 16 who were still ill, the cell count was higher and the total protein content larger than in those who had recovered. As a rule, not only the total protein content and the cell count, but also the clinical picture as well as the immunoelectrophoretic protein pattern improved simultaneously. On this occasion a marked α_2-macroglobulin precipitate was found in 5 cases, β_1-lipoprotein in 3, fibrinogen in 5, γ_1-macro- in 10 and a marked anodic γ_{ss}-globulin precipitate in 5. Of 20 patients who had recovered, only 5 still showed pathologic precipitates in the C.S.F., but of 16 who were still ill, 10 showed a pathological protein pattern at re-examination 6—10 weeks after the onset of symptoms. Among these 15 in whom the immunoelectrophoretic protein pattern was not normal, only 1 belonged to the mumps meningoencephalitis grup. In the other 7 with this diagnosis the pattern had become normal.

Of 28 immunoglobulin precipitates observed at the previous examination, 15 persisted, against only 9 of 28 transprotein precipitates, which shows that it was above all the α_2-macroglobulin, β_1-lipoprotein and fibrinogen that decreased in concentration with the patient's recovery. On comparison of each precipitate with each of the other precipitates in each patient, it was also found that the γ_1-macroglobulin precipitate often persisted longer than the 3 transproteins and

marked anodic γ_m-globulin precipitate. β_1-lipoprotein and marked anodic γ_m-globulin precipitate was as a rule demonstrated in patients with a large total protein content, while γ_1-macroglobulin was demonstrated in 7 of 10 patients with a normal total protein content. It is remarkable that 7 of the patients who showed γ_1-macroglobulin in the C.S.F. also had slight pleocytosis, mainly of mononuclear type.

Eight persons had transproteins in the C.S.F. In 5 of these cases the total protein content was normal. Only in 2 cases were transprotein precipitates found as the only abnormality. On the other hand in 4 cases γ_1-macroglobulin was the only pathological precipitate found. The total protein content was normal in these cases except in 1 who had 53 mg. per 100 ml. γ-globulin fractions in the C.S.F. and serum determined by paper electrophoresis were found to be normal except in 1 case, where the relative γ-globulin concentration in the C.S.F. was 18.1 %. A fifth patient, who also had a normal total protein concentration showed both γ_1-macroglobulin and marked anodic γ_m-globulin precipitate. Marked anodic γ_m-globulin precipitate was found as the only pathologic component in 2 cases with slightly increased total protein concentration and normal γ-globulin fraction in the C.S.F.

In order to form an opinion as to whether the concentration of γ_1-macroglobulin was increased in the serum and the C.S.F. at the same time, the concentration of the γ_1-macroglobulin in serum was roughly estimated by studing the position and appearance of the precipitate. The 10 who had γ_1-macroglobulin in the C.S.F. included 2 whose serum precipitate was judged as +++, 6 as ++ and 2 as +. Of the 26 who showed no γ_1-macroglobulin in the C.S.F., 6 had +++, 7 ++, 8 +. This precipitate was not demonstrated in the serum in 5. Thus, marked γ_1-macroglobulin precipitates in serum were more common among *the patients who had γ_1-macroglobulin in their C.S.F. The degree of* increase in the serum was, however, not substantial enough to be measured by electrophoresis. The absolute concentration of the serum γ-globulin was determined in 30 cases, and in only one was it found to be increased (1.21 g./100 ml.). The relative concentration was measured in 4 cases, and was found to be normal in all of them. In 2 cases the concentration *is not known.*

SUMMARY

Of 116 persons re-examined 6—10 weeks after onset of disease, 89 were discharged as healthy. C.S.F. and serum were analysed from 20 healthy and 16 patients still ill. There was a tendency to a higher cell count and higher total protein content in the group of still ill patients. Of 20 healthy persons 15 had a normal protein pattern in C.S.F. and of 16 patients still ill, 10 had an abnormal protein pattern. γ_1-macroglobulin was the most common finding and was shown in 10 cases. Immunoglobulin precipitates were found as the only pathologic finding even in C.F.S. with normal cell and total protein content. Transprotein precipitates were found in 8 cases. In 5 of these cases the total protein content was normal. In no case a precipitate occurred for the first time in this phase of the disease.

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BACTERIAL MENINGITIS

MATERIAL

The material consisted of 27 cases of bacterial meningitis in which the C.S.F and the serum were studied immunoelectrophoretically The material covers the period from May 1960 to Feb 1964, during which 29 patients were admitted to the department for infectious diseases, University Hospital Lund, because of bacterial meningitis. Seven of these patients were excluded because of coexisting neurological disease such as tumour or cerebral haemorrhage and 1 because of bleeding in association with lumbar puncture. This left 21 cases, to which were added 6 cases from the Children's hospital, Lund, and the department for infectious diseases, Malmö general hospital.

In 3 cases no definite bacteriological diagnosis could be made. In 4 others the aetiology was determined with a high degree of probability and in 20 the diagnosis was confirmed. The patients, of which more than half were children, are summarised below

Table 49 Composition of material

	< 15 years	> 15 years
Meningococcal meningitis	1	8
Pneumococcal meningitis	2	2
Haemophilus influenzae meningitis	5	
Listerial meningitis		1
Tuberculous meningitis	1	4
Purulent meningitis	1	2
	10	17

The large proportion of children may be explained by the relatively higher frequency of analysis of the C.S F in patients at the children's hospital compared with the virus meningo-encephalitis material.

All of the patients except three were examined on *admission, which means that* they had, as a rule, not received treatment before. The samples for the second

analyses were obtained 2—6 days after treatment had been started, and subsequent samples after 10—20 days, i.e. just before the patient was sent home. In a few special cases further specimens were examined. In 4 cases the patients were examined only on admission.

Meningococcal Meningitis Group

Nine patients had meningococcal meningitis. Culture of the C.S.F. gave growth of Neisseria meningococcica in 7 of these cases. The other 2 (Cases 6 and 7) were young men who were doing their military service. Owing to fever and headache they had been treated with a small dose of penicillin for 1 day and 4 days, respectively, which failed to produce the desired effect. On admission culture of the C.S.F. gave no growth, but both of them showed widespread petechiae and purpuric lesions in the skin, i.e. signs of meningococcal sepsis sufficient to suspect meningococcal meningitis.

Two patients were above 20 years and 3 were females. Six patients were admitted in an unconscious state after usually a few day's catarrhal condition, but with acute symptoms of meningitis. Three were somnolent. One patient (Case 9) never recovered consciousness and died 4 days later. The remaining 8 responded favourably to therapy and were sent home after 2—3 weeks.

Results

All the initial C.S.F. samples (Table 50) were obtained on admission, except in one case (No. 2). This patient had been cared for in another department and had received adequate therapy for 2 days before lumbar puncture was done on the 6th day after the onset of symptoms. Apart from this patient all had pronounced pleocytosis, the highest value recorded being 17,800 cells per cu.mm. Most of the cells were polymorphonuclear leukocytes. The total protein content of the C.S.F. was also markedly increased and varied between 147 and 910 mg per 100 ml.

Immunoelectrophoresis showed that in all except the 2 (Cases 2 and 7) who had been treated some days previously, the concentrations of the large molecular plasma proteins α_2-macroglobulin, β_1-lipoprotein and fibrinogen in the C.S.F. were increased. The C.S.F. in Case 7 showed fibrinogen as the only transprotein,

Table 50 Cellcontent, total protein content, γ-globulinconcentration in paper electrophoresis and Imm electrophoretic findings in C.S.F on repeated examinations of 9 patients with meningococcal meningi

N	Sex Age	Day of disease	Cell per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in paper electrophoresis	Marked α_2-macroglobulin	β-lipoprotein	fibrinogen	γ_1-macroglobulin
1	Male 5	2	6000	170	n.	+	+	+	
		6	52	68	n.			+	
		20	10	34	17.4				
2	Male 17	6	410	188	n.	+	+		
		18	22	49	n.				
		60	21	31	n.				
3	Male 17	2	17800	474	n.	+	+	+	+
		8	57	61	n.	+		+	
		21	11	41	n.			+	
4	Female 17	3	4500	342	n.	+	+	+	+
		9	64	67	n.	+			+
		20	1	27	n.				
		63	20	48	n.				
5	Female 19	1	7400	292	n.	+	+	+	+
6	Male 19	2	11700	412	n.	+	+	+	+
		4	2510	132	n.	+			
		14	15	54	n.			+	
		60	35	40	n.				
7	Male 20	5	3300	147	n.			+	+
		8	38	106	18.6			+	+
		19	12	64	18.6			+	
		60	4	42	n.			+	
8	Male 40	1	8000	910	n.	+	+	+	+
		8	75	127	n.			+	
		16	38	93	n.				
9	Female 56	3	6800	485	n.	+	+	+	+
		4	8200	488	n.	+	+	+	+

and the other pretreated patient (Case 2) showed marked α_2-macroglobulin and β_1-lipoprotein precipitates. The fibrinogen precipitates, which were demonstrated in 8 patients, were large and had migrated but little. Of the immunoglobulins, no γ_1-macroglobulin was found in the youngest patient (Case 1) who was 5 years old. γ macroglobulin could not be demonstrated either in one of those who had received antibiotics before the examination (Case 2) The remaining 7 showed large precipitates of γ_1-macroglobulin. All 9 showed marked anodic γ_m-globulin precipitates.

In 5 of the cases the C.S F was re-examined 2—6 days after the beginning of treatment (Cases 1 3 4, 6 7) The cells decreased rapidly in number in

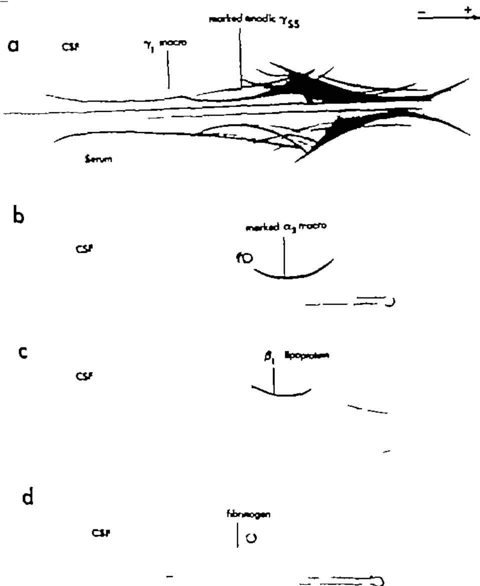
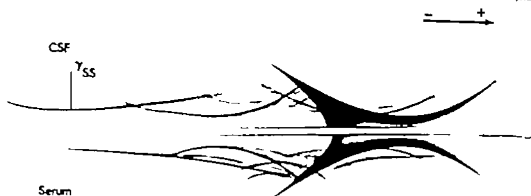


Fig. 3. Immunoelectrophoretic pattern in C.S.F. and serum from case 8 with meningococcal meningitis at onset of disease (cf. table 50). With rabbit [illegible]-antiserum γ-macroglobulin and marked anodic γ-globulin precipitates were demonstrated. b. Monospecific antiserum revealed marked α_2-macroglobulin precipitate. Sudan black staining showed β-lipoprotein. d. Antifibrinogenserum gave fibrinogen precipitate.

a



b



c

CSF

d

CSF

16. Normal immunoelectrophoretic pattern in ? and serum from case 8 with meningococcal ngitis 14 da) after onset of disease (cf. tabl 50)

a. Rabbit-antihuman-antiserum b. A ti-α_2 macroglobulinserum. c. Sudan black staining and abbit antihuman-antiserum. d. Antif bri ogenserum

all of the patients except 1 (Case 6) and consisted, as a rule of about 50 mononuclear cells per cu.mm. The total protein content was only moderately increased except in cases 6 and 7 Immunoelectrophoresis sometimes showed rapid improvement. Two patients (Cases 1 and 6) recovered rapidly In Case 1 the C.S.F showed only fibrinogen. In Case 6 only a marked α_2-macroglobulin precipitate was seen in spite of the fact that he had a total protein content of 152 mg per 100 ml. In case 3 the total protein content fell from 747 to 61 mg per 100 ml. and only marked α_2-macroglobulin precipitate and fibrinogen could be demonstrated. In case 4 and 7 the C.S.F still showed γ_1-macro- and marked anodic γ_A-globulin precipitates.

All except 2 were re-examined 10—20 days after the beginning of treatment. One (Case 9) of them had died. No C.S.F was available in the other case. The remaining 7 had practically recovered and were sent home a few days after this examination. In all of these cases the C.F.S. showed a small number of mononuclear cells. The total protein content had become practically normal except in 1 patient (Case 8) in whom it was 93 mg per 100 ml. (Fig. 15 and 16) Four (Cases 1 2, 4, 8) of the patients had a normal protein pattern of the C.S.F., but in 3 (Cases 3, 6 7) various abnormalities persisted. All 3 had, however atypical precipitation with antifibrinogen serum. One patient (Case 7) who had been admitted with petechial haemorrhages after 4 days unsuccessful antibiotic therapy had after 14 days also a marked anodic γ_A-globulin precipitate in the C.S.F In the serum the γ_A-globulin was also well developed but not more marked than on previous occasions. In this patient also the γ-globulin fraction was found to be slightly increased on paper electrophoresis of the serum and the C.S.F

In 4 cases (Nos 2 4, 6, 7) the patients were re-examined again about 2 months after the onset of the disease. In all of them the total protein content was normal, in 3 the number of mononuclear cells was somewhat increased. In these 3 the protein pattern of the C.S.F was normal. In case 7 however a weak precipitate was still demonstrable with antifibrinogen serum of the same atypical appearance as at the previous re-examination. The marked anodic γ_A-globulin precipitate had, however disappeared from the C.S.F In the serum the γ_A-globulin precipitate was unchanged.

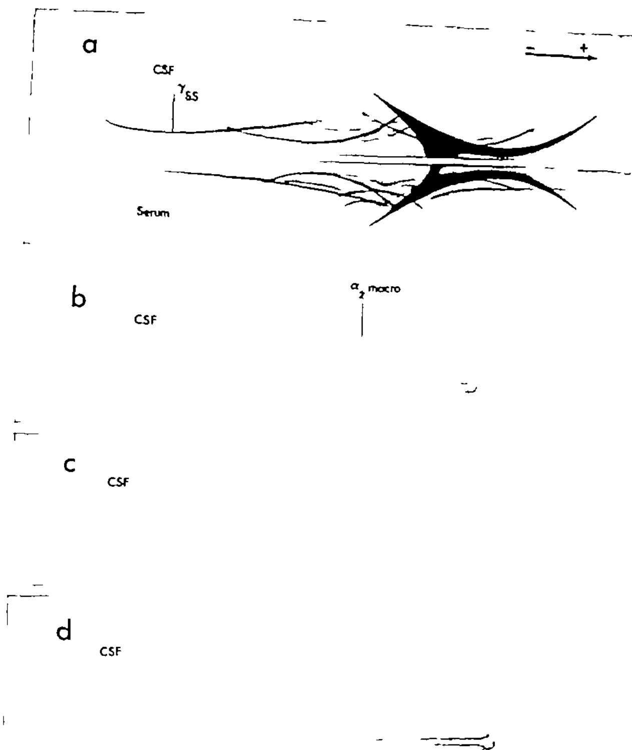


Fig 16. Normal immunoelectrophoretic pattern in C.S.F and serum from case 8 with meningococcal meningitis 14 days after onset of disease (cf table 50) a. Rabbit-antihuman antiserum. b A ti-α_2-macroglobulinserum c. Sudan black staining and abbit antihuman-antiserum. d. Antifibrinogenserum.

le 31 Cell content, total protein content, γ-globulin concentration in paper electrophoresis and immunoelectrophoretic findings in C.S.F. on repeated examinations of 4 patients with pneumococcal meningitis

Sex Age	Day of disease	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in paper electrophoresis	Marked α_2-macroglobulin	β_1-lipoprotein	Fibrinogen	γ_1-macroglobulin	marked γ-globulin
Male 3		1050	60	n.	+	+	+	+	+
	5	83	6	8.4			+		
	1	9	44	n.			+		
	22	7	36	n.			+		
Male 3		5500	74	n.	+	+		+	+
Female 39		2490	76	4.2	+	+	+		
	3	9 60	122	n.	+	+	+		
	9	5	56	n.	+	+	+		
	7	5	44	n.	+		+		
	60	3	35	n.			+		
	20		30	n.			+		
Male 78	3	500	54	8.0	+	+	+	+	+
	6	300	364	10.0	+	+	+	+	+

Immunoelectrophoresis still showed a marked α_2-macroglobulin precipitate af 2 weeks' treatment as well as an atypical fibrinogen precipitate. On re-examir tion 2 respectively 4 months after the onset of the disease this precipitate w still demonstrable.

Haemophilus Influenzae Meningitis Group

Five children, all below 10 years, had meningitis due to Haemophilus influenz The culture of the C.S.F. gave growth of the bacteria in 4 of them. The patient (Case 16) had maxillary sinusitis caused by Haemophilus influenz During treatment with α-aminobenzyl-penicillin (Doktacillin®) the patient ill with purulent meningitis. Culture of the C.S.F. on that occasion gave growth. It is nevertheless likely that also the purulent meningitis had be caused by Haemophilus influenzae. Another patient (Case 15) was given pe cillin and streptomycin 12 hours before the first sample of the C.S.F. was c tained. Another patient (Case 17) was given penicillin 2 days before admissi These 5 patients were in a moderately poor general condition with marked ne

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Pneumococcal Meningitis Group

Four patients had pneumococcal meningitis. Culture of the C.S F gave growth of diplococcus pneumonia in all. All had some co-existing disease. One (Case 13) of them had rheumatoid arthritis and was treated with corticosteroids at the onset of the disease. He was admitted in a poor general condition and died 6 days later One patient (Case 12) had a known hypo γ-globulinaemia with low vital resistance. She was admitted in an unconscious state after 1 day's mild symptoms and left hospital symptomfree after about 3 weeks. The 2 children had had purulent meningitis after head injury 4 respectively 5 years previously One of them (Case 11) who was placed in a respirator died 5 days later The other patient left hospital 3 weeks after admission.

Results

As is apparent from the table, one patient (Case 13) had only moderate pleocytosis on admission. The other 3 had pleocytosis with 1000 to 5 500 mainly polynuclear cells per cu.mm. The total protein content varied between 62 and 540 mg per 100 ml. Immunoelectrophoresis of the C.S.F demonstrated all 5 plasma proteins in all except 2 cases (Nos 10 13) In case 12 the concentration of the immunoglobulins in the C.S F., was, as expected, not increased, but marked α_2-macroglobulin precipitate, β_1-lipoprotein and fibrinogen were noted In case 11 all the pathological precipitates were found except fibrinogen. The examination with antifibrinogen serum was done in this case with varying proportions of antigen and antibody but on no occasion was any precipitate demonstrable.

Only 2 patients (Cases 10 13) were re-examined after 2—6 days treatment. It was then found that in the patient with rheumatoid arthritis (Case 3) pleocytosis had increased after withdrawal of steroid therapy The total protein content was still markedly increased Paper electrophoresis showed an increase of the γ-globulin concentration in the C.S F Neither did the immunoelectrophoretic protein pattern of the C.S F show any signs of improvement. The other patient (Case 10) made a rapid recovery The pleocytosis soon disappeared and the protein pattern became normal with the exception of a slightly atypical fibrinogen precipitate. This precipitate could still be demonstrated 3 weeks later just before the patient left hospital.

The patient with hypo γ-globulinaemia (Case 12) also made a rapid recovery The cell count and the protein content became practically normal after a week.

protein content of the C.S.F. was still increased and varied between 79 and 152 mg. per 100 ml. Immunoelectrophoresis now showed a normal protein pattern in 1 patient (Case 15) in whom the total protein content had fallen from 332 to 152 mg. per 100 ml. On later re-examination, however, a weak precipitate was demonstrated with antifibrinogen serum. In Case 17 γ_1-macroglobulin could no longer be demonstrated but in Case 18 the protein pattern was unchanged, i.e. the marked α_2-macroglobulin precipitate and the fibrinogen were still demonstrable in the C.S.F.

At the examination performed 10—20 days after treatment had been started, 3 patients (Cases 16, 17, 18) were re-examined. In 2 of them (Cases 16 and 17) the protein pattern of the C.S.F. was normal. The cell count and the total protein concentration were also normal or slightly increased.

In Case 18 on the other hand, a substantial amount of large plasma proteins was seen despite a practically normal total protein content of the C.S.F. It is noteworthy that the γ_1-macroglobulin occurred in this stage of the disease during antibiotic therapy. Marked anodic γ_m-globulinprecipitate was, however, missing. This patient was re-examined 2 respectively 4 months after onset of symptoms. The concentration of the total protein was normal. The C.S.F. still contained a few mononuclear cells, and immunoelectrophoresis of the C.S.F. showed γ_1-macroglobulin, but not any marked anodic γ_m-globulin precipitate. γ_1-macroglobulin was identified in all of the serum samples. The concentration of the precipitate, as judged from its position and appearance, did not vary with certainty during the disease. As judged by paper electrophoresis, the concentration of the γ-globulin was almost normal in the C.S.F. and normal in the serum.

Listerial Meningitis Group

One patient had listeral meningitis. The diagnosis was confirmed by culture of the C.S.F. and serological examination. The patient was admitted in a moderately poor condition a few days after the onset of symptoms. He responded well to antibiotics, made a complete recovery and left hospital 5 weeks after admission.

The patient had the purulent form of listeral meningitis with about 1 500 mainly polynuclear cells per cu.mm. in the C.S.F. The total protein content was

Table 52 Cellcontent, total protein content, γ-globulin concentration in paper electrophoresis an munoelectrophoretic *findings in C.S.F* on repeated examinations of 5 patients with haemophilus meningitis

	Sex Age	Day of disease	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in paper electrophoresis	Marked α_2-macroglobulin	β_1-lipoprotein	Fibrinogen	γ_1-macroglobulin
14	Male 1	2	5960	154	n.	+	+	+	
15	Female 3	4	24800	332	n.	+	+	+	
		6	1330	152	n.				
		11	76	60	n.			+	
16	Male 7	1	3000	170	n.	+	+		+
		15	8	34	n.				
17	Male 8	3	2300	78	n.	+		+	+
		5	550	79	n.	+		+	
		19	15	38	n.				
18	Female 9	3	3000	156	n.	+		+	
		5	2600	159	n.	+		+	
		14	57	55	15.4	+		+	+
		60	6	27	n.				+
		120	12	40	15.1				+

stiffness but their level of consciousness appeared to be normal. The other 2 pa tients who were not treated with antibiotics at the onset of symptoms were admitted in a very poor condition with loss of consciousness and pronounced neck stiffness. Adequate treatment produced the desired effect and all the patients left hospital 2—3 weeks later

Results

At examination on admission, the number of cells, mainly polynuclear cells, in the C.S F was invariably increased with peak values of 24,800 cells per cu.mm. The total protein content of the C.S F was, as a rule, increased and varied between 78 and 332 mg per 100 ml.

Immunoelectrophoresis showed that none of the patients had all 5 plasma proteins in the C.S.F Marked α_2-macroglobulin precipitate was demonstrated in all the patients and fibrinogen in 4. Only 3 of the patients had β_1-lipoprotein in any demonstrable amount in the C.S.F The increased concentra tion of the immunoglobulins was entirely missing in 3 while γ-macro- and marked anodic γ_{2}-globulin precipitates were demonstrated in 2

In 3 cases (Nos 15 17 18) the second examination was done 2 days *after* commencement of treatment. Two of *the patients still had pleocytosis. The total*

protein content of the C.S.F. was still increased and varied between 79 and 152 mg. per 100 ml. Immunoelectrophoresis now showed a normal protein pattern in 1 patient (Case 15) in whom the total protein content had fallen from 332 to 152 mg. per 100 ml. On later re-examination, however, a weak precipitate was demonstrated with antifibrinogen serum. In Case 17 γ_1-macroglobulin could no longer be demonstrated but in Case 18 the protein pattern was unchanged, i.e. the marked α_2-macroglobulin precipitate and the fibrinogen were still demonstrable in the C.S.F.

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The patient had the purulent form of listerial meningitis with about 1,500 mainly polynuclear cells per cu.mm. in the C.S.F. The total protein content was

Table 53 Cellcontent, total protein content, γ-globulin concentration in paper electrophoresis and immunoelectrophoretic findings in C.S.F on repeated examinations of 1 patient with bacterial meningitis

Nr	Sex	Age	Day of disease	Cells per cu. mm.	Total protein mg. per 100 ml.	γ glob. conc. in paper electrophoresis	Marked γ_M-macroglobulin	β-lipoprotein	Fibrinogen	γ_1-macroglobulin
19	Male	55	3	1370	150	n.			+	
			5	320	115	n.				
			10	76	140	n.				
			17	59	74	n.				
			30	10	89	n.				

130 mg per 100 ml. Immunoelectrophoresis showed only a marked fibrinogen precipitate. On re-analysis a few days after treatment had been started the cell count had fallen considerably and the total protein concentration was 115 mg per 100 ml. Immunoelectrophoresis no longer demonstrated fibrinogen and the protein pattern was of normal appearance. Five days later the C.S.F was examined again. The number of cells had now fallen to 76 per cu.mm. the total protein content was 140 mg per 100 ml. and immunoelectrophoresis showed a marked anodic γ_M-globulin precipitate in the C.S.F. Repeated examinations during the following month showed a successive decrease of the total protein content and the cell count, but all samples showed marked anodic γ_M-globulin precipitates. The serum γ_M-globulin precipitates did not differ substantially from one occasion to another. They were relatively marked throughout. The paper electrophoresis of the C.S.F and serum showed no increase of the γ-globulin concentration.

Tuberculous Meningitis Group

Five patients had tuberculous meningitis. In 4 cases the diagnosis was confirmed by culture and inoculation of the C.S.F into guinea pigs. Similar examination of the C.S.F in 1 patient (Case 21) proved negative. But only 1 sample was cultured before specific treatment had been started. There is reason to believe that also this patient had tuberculous meningitis, for she had been operated upon in 1945 for renal tuberculosis. The onset of the symptoms was typical of tuber

culous meningitis with insidious symptoms of meningitis. The examinations of the C.S.F before treatment repeatedly showed a decrease in concentration of chlorides and sugar All the cells were also mononuclear The patient left hospital after 2 months' specific treatment, which was afterwards continued at the out patient department. Two patients (Cases 20 22) died from miliary dissemination 5—10 days after admission. The remaining 2 (Cases 23, 24) were not examined in the beginning of the disease but first after 2 1/2 respectively 4 months.

Results

It is clear from the table that in the beginning of the disease 3 patients examined (Cases 20, 21 22) had moderate pleocytosis with a cell count varying between 111 and 425 per cu.mm. Most of the cells were mononuclear The total protein content in 2 cases was markedly increased with peak value of 194 mg. per 100 ml. Immunoelectrophoresis revealed marked α_2-macroglobulin precipitate and β_1-lipoprotein in the C.S.F in all, while fibrinogen was seen in only one (Case 20) of them. Despite varying proportions of C.S.F concentrate and antiserum no fibrinogen precipitate could be demonstrated in Cases 21 and 22 in this stage of the disease. All 3 had marked anodic γ_2-globulin precipitates but in none was any γ_1-macroglobulin demonstrable although they were identified in the serum.

On re-examination some days later 2 patients (Cases 21 22) who could be followed up were found to have a lower cell count, but the total protein content

…nt, total protein content, γ-globulin concentration in paper electrophoresis and im-
…ic findings in C.S.F on repeated examinations of 5 patients with tuberculous meningitis

Day of disease	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in paper electro-phoresis	Marked α_2-macro-globulin	β_1-lipo-protein	Fibri-nogen	γ_1-macro-globulin	marked anodic γ-globulin
9	4 5	13	6.	+	+	+		+
3	180	60	n.	+	+			+
	60	33	n.	+	+	+		+
24	88		n.		+			+
48	8	77	n.		+	+		
90		51	n.		+	+		
80		43	n.			+		
8		94	n.	+	+			+
6	44	137	6.5	+	+	+		+
75	83	300	n.	+	+	+		+
10	4	58	n.					

had increased. In both patients fibrinogen was demonstrable on this occasion. The precipitate in 1 case was situated near the starting point, but in the other the antigen had migrated in the electric field. γ_1-macroglobulin could not be demonstrated on this occasion either. Marked α_2-macroglobulin, β_1-lipoprotein and marked anodic γ_M globulin precipitates were demonstrable in these cases as on admission.

In Case 21 the patient was re-examined on various occasions during the first 6 months. The cell count and the total protein content of the C.S.F. became normal successively as did the appearance of the protein pattern. Marked anodic γ_M-globulin precipitate could not be demonstrated in the C.S.F. for more than one month. β_1-lipoprotein was found in weak concentration even 3 months after onset. A weak precipitate with antifibrinogen was still found at examination after 6 months and this precipitate was situated centrally.

Of the 2 patients who were examined later in the course of the disease in Case 24 the total protein content was largely normal and the protein pattern was normal. In the other (Case 23) the total protein content was 300 mg. per 100 ml., the cell count was 83 per cu.mm. and immunoelectrophoresis showed all plasma proteins studied except γ_1-macroglobulin. Unfortunately this patient was not examined again later.

Purulent Meningitis Group

In 3 cases of bacterial meningitis it was not possible to type the causal agents. These cases were classified under the heading of purulent meningitis. Two of them (Cases 25, 27) had been treated with antibiotics, 1 respectively 3 days before the first sample of the C.S.F. had been obtained.

Results

Case 25 had severe pleocytosis. The total protein content in Cases 25 and 27 was about 250 mg. per 100 ml. but the immunoelectrophoretic protein pattern was very slightly changed. Case 25 showed only a marked α_2-macroglobulin precipitate and fibrinogen. Case 27 showed only fibrinogen. On re-examination during and after treatment the immunoelectrophoretic pattern was normal in both cases.

ellcount, total protein content, γ-globulin concentration in paper electrophoresis and im-
phoretic findings in C.S.F. on repeated examinations of 3 patients with purulent meningitis

	Day of disease	Cells per cu. mm.	Total protein mg. per 100 ml.	γ-glob. conc. in paper electro-phoresis	Marked α_2-macro-globulin	β_1-lipo-protein	Fibri-nogen	γ_1-macro-globulin	marked anodic γ_2-globulin
	3	32000	243	n.	+		+		
	8	77	118	n.					
	36	61	4	n.					
	60	5	36	n.					
61	4	29200	840	n.	+	+	+	+	+
	9	737	203	n.		+	+		
	16	343	33	n.			+		
	26	37	33	5.6	+	+	+		+
	33	7	39	3.4				+	+
	120	37		23.8				+	+
	2	4	70	n.					+
n 64		745	30	n.			+		
		8	63	n.					

The third patient (Case 26) fell ill with fever headache and neck-stiffness within a few days, for which he was treated with phenylbutazonnatrium (Butazolidin®) On admission the patient was severely ill and the following day he lost consciousness and was treated for some days in a respirator After 14 days treatment with antibiotics, the body temperature was normal and the patient recovered consciousness. During this period the C.S.F had also improved. The cell count fell from 29,200 to 343 per cu.mm. The cells were still mainly polynuclear The total protein content fell from 840 to 153 mg. per 100 ml. The immunoelectrophoretic protein pattern of the C.S.F became practically normal. Only a central fibrinogen precipitate persisted. The concentration of the immunoglobulins was no longer increased.

A new bout of fever presumably not due to any disorder of the C.N.S., for the cell count fell successively but to sulphatherapy which was also held responsible for erythrodermia supervened and lasted from the 15th and 36th day of the disease. The cell count had thus decreased, while the total protein content was still high. During this period a change was noticed in the protein pattern. Marked α_2-macroglobulin precipitate and β_1-lipoprotein recurred. Marked anodic γ_2-globulin precipitate was demonstrated after having disappeared for 2—3 weeks.

On repeated controls during convalescence, in the course of which the patient recovered only slowly the total protein content and the cell count decreased,

but the γ-globulin concentration in the C.S.F measured by paper electrophoresis still showed a relative increase. Immunoelectrophoresis one month respectively 4 months after the onset of the disease again showed γ_1-macroglobulin and marked anodic γ_{ss}-globulin precipitates as the only pathological findings. The latter was the only precipitate found at the last examination 7 months after the onset of the disease, one month after the patient had gone back to work.

Immunoelectrophoresis of the serum in this case invariably showed γ_1-macroglobulin except at re-examination after about 14 days antibiotic therapy As in the C.S F., this protein reappeared in the serum in association with clinical deterioration. The serum precipitates were as a rule weaker than those of the C.S F The γ_{ss}-globulin precipitates in the serum were always marked and of the same appearance as in the C.S.F Judging from the paper electrophoretic pattern, the serum γ-globulin concentration was always normal except on one occasion.

Discussion

The initial phase of bacterial meningitis is characterised by severe pleocytosis and large total protein content of the C.S F Exceptions to this rule are *inter alia* tuberculous meningitis and some cases of listerial meningitis which may occur in the serous form. According to SCHÖNENBERG (1960) pleocytosis and the increase of the total protein content of the C.S.F vary with the severity of the inflammation. The total protein content and the cell count reach higher values in pneumococcal meningitis than in meningococcal and haemophilus infection (SCHÖNENBERG 1960 CARPENTER and PETERSDORF 1962) The present material was not large enough to warrant such comparisons. Severe pleocytosis was sometimes seen in relatively mild cases of the disease. The peak value found was 32 000 cells per cu. mm. In 4 it was only 500 cells per cu.mm. or fewer at the onset of the disease. Three of them however had tuberculous meningitis. One was treated with corticosteroids because of rheumatoid arthritis and therefore less able to withstand infections.

The total protein content was very high with 284 mg per 100 ml. on the average. The highest value noted was 910 mg per 100 ml. Three patients had less than 100 mg per 100 ml.

As indicated by the total protein content, the disorder of the *blood-C.S.F*

Table 56 Frequency of pathologic precipitates in C.S F in virus meningoencephalitis and bacterial meningitis

Diagnosis	N	marked α_2-macroglobulin per cent	β_1-lipoprotein per cent	fibrinogen per cent	γ_1-macroglobulin per cent	marked anodic γ_{2a}-globulin per cent
Virus meningoencephalitis	3	51	38	56	4	9
Bacterial meningitis	7	83	76	80	52	72

barrier was more severe in bacterial meningitis than in virus infections. This was reflected also in the results of the immunoelectrophoresis of the C.S.F The large molecular plasma protein, which are normally prevented from passing into the C.S.F space, occurred in the C.S.F more often and in higher concentrations in bacterial infections. In none of the patients was the C.S.F protein pattern normal, which does sometimes happen in virus infections of the C.N.S.

Large serum proteins were, however seen not only in the C.S.F where the total protein content was very much increased, but also in cases before the massive passage of smaller protein molecules into the C.S F space. Thus 3 patients had moderately elevated values, the highest being 78 mg per 100 ml., but none the less most of the large plasma proteins were demonstrated in the C.S.F

Another difference between bacterial and virus infection of the C.S.N was, that marked anodic γ_{2a}-globulin precipitate occurred more often than γ_1-macroglobulin in bacterial meningitis, presumably owing to a more ready passage into the C.S.F of γ_{2a}-globulin molecules because of the lower molecular weight. As in virus meningoencephalitis, immunoglobulin precipitates were demonstrated more often in the C.S.F in adults than in children. Transproteins appeared to be equally common in the 2 age groups. There might have been a tendency as in virus meningoencephalitis, for the α_2-macroglobulin precipitate to occur substantially more often in children.

Specific treatment of bacterial meningitis will rapidly control pleocytosis. Also the total protein content rapidly decreases, though somewhat slower In 13 patients who had different forms of bacterial meningitis, but not tuberculous meningitis the C.S F was examined 2—6 days after treatment had begun. In as many as 6 of them the cell count was less than 100 per cu.mm. Four still had more than 1,000 cells per cu.mm. but 3 of the last mentioned 4 were examined only after 2 days treatment. The total protein content had fallen to less than 100 mg per 100 ml., in 3. On immunoelectrophoresis 3 persons had recovered a normal protein pattern though the total protein content exceeded 100 mg. per 100 ml. Of the

remaining 10 patients marked α_2-macroglobulin precipitates were found in 6 and fibrinogen in 8. Of 8 who showed β_1-lipoprotein on admission, only 2 showed this fraction after some days treatment. Of 8 who showed γ macroglobulin and marked anodic γ_{ss}-globulin precipitate on the first occasion, this re-examination showed these two immunoglobulins in only 3 and marked anodic γ_{ss}-globulin precipitate in a fourth. Thus already within a few days' treatment the protein pattern improved. As at re-examination of cases of virus meningoencephalitis, β lipoprotein was demonstrated least often, probably because it has a higher molecular weight than the other 4 proteins studied. In contrast to what was found in virus meningoencephalitis, where the number of immunoglobulin precipitates decreased only slightly at the re-examination, the corresponding reduction here was substantial. In none of the cases was any precipitate demonstrated that had not been found at the first examination on admission.

At re-examination 10—20 days after the onset of the disease 15 persons except those with tuberculous meningitis were examined. Ten of them had also been examined after a few days' treatment. Most of the patients still had 10 or more mononuclear cells in the C.S.F. Only 1 had more than 100 cells per cu.mm. The total protein content had fallen below 50 mg per 100 ml. in 8 and 1 had more than 100 mg per 100 ml. As many as 7 showed a normal protein pattern and together with the results of the previous re-examination it was found that in 10 of 18 patients continuously studied the pattern had become normal within the first 20 days. Of the remaining 8 patients who still showed a pathologic protein pattern after 10—20 days 7 showed precipitates with antifibrinogen serum and only one of them had a normal appearance. Two patients had both a marked α_2-macroglobulin precipitate and fibrinogen.

The precipitates which were obtained with antifibrinogen serum at the re-examinations generally differed from those seen at the onset of the disease and in virus meningoencephalitis. The precipitates were weak and consisted of 2 or 3 determinants, which migrated in the electric field and were situated some distance from the hole of origin. The picture agreed with that found for precipitation with fibrinolytic products. (BURTIN 1960 NUSSENZWEIG and SELIGMANN 1960) These precipitates were presumably a manifestation of the breakdown of the fibrin from the acute phase of the disease rather than of the continued passage of fibrinogen into the C.S.F space. This assumption is supported by the observation of QUAADE and KRISTENSEN (1961) On patho-anatomic examination of 21 patients who had died from complications of bacterial meningitis treated with antibiotics from 2—16 days, they found thick layers of fibrin covering the convexity of the brain.

Three patients in the second re-examination showed signs of increased immunoglobulin concentration in the C.S.F for a long time despite intense antibiotic therapy In 1 case both paper and immunoelectrophoresis showed the increase for 7 months. The increase in the γ-globulin in all 3 cases recurred after some weeks of the disease, as in virus meningoencephalitis, in spite of therapy In 1 case a possible correlation was found with the clinical picture since this patient did not recover in the usual way In 2 of the cases the total protein content was increased, but no other pathological plasma proteins were found in the C.S.F The paper as well as the immunoelectrophoretic pattern of the serum was normal.

The aetiological groups are too small to warrant any comparison. The patient with Listerial meningitis differed distinctly from the others. In the initial stage of the disease he had a large total protein content of the C.S.F suggesting a severe disorder of the blood-C.S.F barrier But the disorder did not appear to be so profound as in other cases of bacterial meningitis, for the only abnormal precipitate found in the C.S.F was fibrinogen. Repeated examination failed to reveal any of the other large molecular plasma proteins. In no other case of untreated bacterial meningitis with a total protein content of the same order did the immunoelectrophoretic pattern differ so little from normal.

The most striking thing in the evaluation of patients with tuberculous meningitis is that none had γ_1-macroglobulin in the C.S.F In spite of other signs of severe disorder of the blood-C.S.F barrier γ_1-macroglobulin did not pass into the C.S.F space in demonstrable amounts. This protein was demonstrable in the serum but with fairly weak precipitates, which may be in line with the observation of BURRELL et al. (1956) that precipitines against the tubercle polysaccarides are 7 S γ-globulins. All 3 patients who were examined before treatment was started had a marked anodic γ_{a}-globulin precipitate. The patient who could be re-examined on various occasions had marked anodic γ_{a}-globulin after 1 month s treatment. During further follow-up no signs of marked anodic γ_{a}-globulin precipitate were seen, but a fibrinogen precipitate at its ordinary site was demonstrated 6 months later The patient, who was examined for the first time after 2 1/2 months treatment still had increased total protein content of the C.S F and marked anodic γ_{a}-globulin precipitate as well as large transproteins were demonstrated. Since increased fibrinogen concentration in C.S.F is regarded as pathognomonic of tuberculous meningitis, it is noteworthy that fibrinogen was not demonstrable in 2 patients in the initial stage of the disease. At re-examination about one week later however fibrinogen was demonstrated in both. In one of them it was seen in the form of an atypical precipitate.

It is possible that a characteristic development of the protein pattern of the

C.S.F. during normalization after bacterial infection should be as follows. The blood-C.S.F. barrier gradually recovers and large serum proteins can no longer pass into the C.S.F. space. The protein concentration of the C.S.F. is then still high as a sign of an increased passage of small molecular proteins. Complete normalization does not occur until later and is manifested by the concentration of the total proteins to normal (Case 8). There are, however, some exceptions from this characteristic development, *e.g.* persistent atypical fibrinogen precipitate and supervening immunoglobulin precipitate.

It is difficult to decide whether concentration of the serum immunoglobulin increased simultaneously in these 3 cases with supervening immunoglobulin precipitates in the C.S.F. In order to find out whether these precipitates were due to immunoglobulins that had passed over into the C.F.S. an analysis was made of the immunoelectrophoretic pattern as a whole. It was found that in this stage of the disease no transproteins could be demonstrated in the C.S.F. in any of these 3 cases of bacterial meningitis. This strongly suggests that the increase in the concentration of the immunoglobulins was not due to any increased passage of plasma proteins but to intrathecal production of the immunoglobulins.

Summary

In 27 patients with bacterial meningitis the C.S.F. and serum was studied immunoelectrophoretically on various occasions during the course of the disease. Twenty four of the patients were examined in the acute stage of the disease. Marked α_2-macroglobulin precipitates were found in 88 %, β_1-lipoprotein in 76 %, fibrinogen in 80 %, γ_1-macroglobulin in 52 % and marked anodic γ_2-globulin precipitate in 72 % of the cases. Such plasma proteins were also demonstrated in cases with only a slight increase of the total protein content of the C.S.F. Immunoglobulin precipitates were found more often in adults than in children.

Therapy was, as a rule, accompanied by a tendency to rapid improvement of the protein pattern. Of 13 patients re-examined within 2—6 days the pattern was found to be normal in 3. At re-examination of 15 patients after 10—20 days the pattern was normal in 7. Thus, in all together 10 of 18 patients with bacterial meningitis without tuberculosis the C.S.F. pattern became normal within 3 weeks. The protein pattern often became normal before the total protein con

tent. Of the remaining 8 with an abnormal immunoelectrophoretic protein pattern, 6 showed an atypical precipitate with antifibrinogen serum, which might be a manifestation of the breakdown of fibrinogen from the acute phase of the disease rather than of the continued passage of fibrinogen into the C.S.F. space. In one case of tuberculous meningitis, however, a typical fibrinogen precipitate was demonstrated after 6 months.

At the re-examination 3 patients showed immunoglobulin precipitates which had not been demonstrable at the preceding investigations. This suggests an intrathecal production of immunoglobulins rather than an increased passage from the serum. In one case γ_1-macroglobulin was noted as late as 4 months and a marked anodic γ_{1A}-globulin precipitate 7 months after the onset of the disease.

DISCUSSION AND CONCLUSIONS

The purpose of the investigation was to study the immunoelectrophoretic protein pattern of the C.S.F. and of the serum in various infections and to correlate the findings with clinical data. As expected from previous investigations, the protein pattern of the C.S.F. was found to differ from that of the serum. The main difference was that the immunoelectrophoretic pattern of the C.S.F. showed none of the proteins except α_2-macroglobulin with a molecular weight of more than 150 000—200 000 and normally occurring in the plasma. This means that immunoelectrophoresis did not show any β_1-lipoprotein, γ_1-macroglobulin or fibrinogen in normal C.S.F. The rapidly migrating γ_{ss}-globulin molecules, which form the anodic part of the γ_{ss}-globulin band situated in the α_2 and β_1-regions are also missing. The α_2-macroglobulin can normally be demonstrated in some C.S.F. samples in only very low concentration. C.S.F. and, with but few exceptions, the serum from 131 patients with various forms of acute virus meningoencephalitis were studied. Of these patients, 77 were re-examined 2—3 weeks, and 36 of them also again 6—10 weeks, after onset of the disease. In addition, C.S.F. from 27 patients with different forms of bacterial meningitis were studied in the course of the disease. The purpose of the examination was to find out to what extent marked α_2-macroglobulin, β_1 lipoprotein, fibrinogen, γ_1-macroglobulin and marked anodic γ_{ss}-globulin precipitates could be demonstrated in the C.S.F.

The term *blood-C.S.F. barrier has long been used to designate a hypothetical* barrier separating the blood from the C.S.F. and thereby explaining the difference between the composition of the blood and that of the C.S.F. The anatomical basis of this barrier and its physiological function are not known with certainty, but the barrier is widely believed to serve as a semipermeable membrane preventing *among other* things, the passage of large molecular plasma proteins into the C.S.F.-containing space.

To indicate their possible difference from the two immunoglobulins (γ_1 macroglobulin and γ_{ss}-globulin) α_2-macroglobulin, β_1-lipoprotein and fibrinogen were

called transproteins, i.e. they had certainly passed from the blood into the C.S.F containing space. In the beginning of the disease, when the severity of the disorder of the blood-C.S.F barrier is greatest, the protein pattern of the C.S.F was abnormal in 83 % of the patients with virus meningoencephalitis. The occurrence of all 5 plasma proteins in the C.S.F., i.e. a sign of a severe disorder of the blood-C.S.F barrier was noted more often in patients with bacterial meningitis than in patients with virus meningoencephalitis. In contrast to what was seen in virus meningoencephalitis, the protein pattern was never found to be normal in bacterial meningitis. The duration of the disorder of the blood C.S.F barrier was studied in a number of patients examined during their illness and after the clinical symptoms had disappeared. At examination 2—3 weeks after onset of the disease the protein pattern was still abnormal in 60 %. If however the function of the barrier is judged solely from the passage of transproteins, it was impaired in only 31 %. At re-examination 6—10 weeks after the onset of the disease, by which time most of the patients were symptomfree, the protein pattern was still abnormal in as many as 41 % but if as above, only the transproteins be considered, in only 25 %. The bacterial meningitis series was not large enough to warrant comparison. Thirteen of the patients were re-examined after only a few days' treatment and in 3 the protein pattern was found to be normal though the total protein content was increased. At re-examination after 10—20 days' treatment the protein pattern was normal in a further 7 i.e. in all together 10 of 18 examined. Transproteins could still be demonstrated in the C.S.F of 7 of these remaining 8 patients. Eight subjects were re-examined 2 months after onset of the disease. In 2 of them precipitates were obtained with antifibrinogen serum. Fibrinogen precipitate when demonstrated in a later stage of bacterial meningitis, often had an atypical shape, and was probably a sign of breakdown of fibrin from the earlier stage of the disease and not of continued passage of fibrinogen into the C.S.F -containing space.

As to the production of antibodies in the C.S.F -containing space, investigation of the immunoglobulins in different stages of both groups of diseases revealed the occurrence of immunoglobulin precipitates more often in the beginning of the disease than in the later course. But the decrease in the number of immunoglobulin precipitates was smaller than in that of the transprotein precipitates with the result that γ_1-macroglobulins were the commonest findings at both re examinations in virus meningoencephalitis. Of 55 patients who showed γ_1-macroglobulin and 22 who showed marked anodic γ_a-globulin precipitat 17 and 9, respectively also showed these precipitates 2—3 weeks after the onset of the disease. Of still greater interest, however was the fact that γ-macroglobulin

made its first appearance in the C.S.F in 12 patients, and marked anodic γ_m-globulin in 8 patients, at re-examination 2—3 weeks after onset of the disease. The increase of the γ-globulin *concentration of the* C.S.F was, as a rule, not accompanied by any increase in the concentration of the γ-globulin in the plasma. When transproteins occurred they usually did so at the beginning of the disease, rarely did they make their first appearance at re-examination 2—3 weeks after onset. Ten of 16 patients who showed γ-macroglobulin 2—3 weeks *after onset* of the disease and 5 of 12 who showed marked anodic γ_m-globulin also showed these precipitates at re-examination 6—10 weeks after onset.

In patients with bacterial meningitis re-examination after treatment had been started showed only a few immunoglobulin precipitates. The occurrence of precipitates in the beginning was presumably a manifestation of a severe disorder of the blood-C.S.F barrier In 3 cases, however immunoglobulins were found later in the *course of the disease. In 1 case γ_1-macroglobulin was demonstrated* 4 months, and in another case marked anodic γ_m-globulin precipitate 7 months, after onset of the disease. It is noteworthy that in 4 cases of active tuberculous meningitis the immunoelectrophoresis showed γ_1-macroglobulin in the serum but not in the C.S.F In both groups of disease immunoglobulin precipitates were more common in children than in adults. In bacterial meningitis, marked anodic γ_m-globulin precipitates occurred more often than γ macroglobulin in the C.S.F., probably because of the smaller size of its molecules. It is probable that disorders of the blood-C.S.F barrier do not play the same role in virus meningoencephalitis as in bacterial meningitis. In the former disease γ_1-*macroglobulin was demonstrated more often, which may reflect an* early production of antibodies. For it is known that during different phases of the immunisation different globulins are the carriers of antibody function. Experimental and clinical investigations with bacteria and with virus as antigen in man have shown that γ_1-macroglobulin antibodies are the first to be formed during the actual immunisation process and that this formation is followed by the production of 7 S γ-antibodies. (FINK et al. 1962 LO SPALUTTO et al. 1962, WIEDERMANN et al 1963 SMITH and EITZMAN 1964) The results show that even in the benign acute infections of the C.N.S described as well as in such chronic disease as multiple sclerosis and subacute demyelinating leukoencephalitis, there is a solitary increase *of the γ-globulin concentration* in the C.S.F and that there is strong reason to assume that this antibody is formed intrathecally

In order to assess the practical value *of the method, the immunoelectrophoretic* pattern was studied in patients with a normal total protein content. In infections

of the C.N.S. the blood-C.S.F barrier is more or less severely disturbed with consequent escape of plasmatic fluid into the C.S.F-containing space. This is, however not always reflected in an abnormal increase in the concentration of the total protein in the C.S.F In many cases no increase in the concentration is demonstrable either because the increase, if any is too small to be recorded by conventional methods or possibly because the increased value does not fall outside the normal range of variation of the total protein concentration. At onset of the disease the total protein concentration was normal in 43 of 131 subjects studied, but as many as 33 cases (77 %) showed some abnormality of the protein pattern. Of 77 subjects examined 2—3 weeks after onset, 38 showed a normal total protein content. Eleven (29 %) of them showed, however abnormalities of the protein pattern. 6—10 weeks after onset the total protein concentration was normal in 22, and in 9 (40 %) of them the protein pattern was abnormal.

Thus, despite a normal total protein content (≤50 mg. per 100 ml.) at 103 examinations an abnormal protein pattern, i.e. an objective sign of disease in the C.N.S., was seen at as many as 53. In bacterial meningitis the total protein content is rarely normal in the acute stage, and immunoelectrophoresis therefore less useful in this respect. Three patients, however had moderately elevated values, the highest being 78 mg. per 100 ml., but none the less most of the large plasma proteins were demonstrated in the C.S.F At re-examination 10—20 days after treatment had begun the total protein content had fallen below 50 mg per 100 ml. in 8 In this group 2 fibrinogen precipitates and 2 marked α_2-macroglobulin precipitates were demonstrated.

The method has also proved useful in the estimation of the severity of meningoencephalitis. At onset all of the 5 plasma proteins studied were more common among the severe than among the mild cases. The difference in frequency between fibrinogen and α_2-macroglobulin was, however only small. But β_1-lipoprotein and the two immunoglobulins were more common in severe cases. The higher frequency of pathologic precipitates among the severe cases was not due entirely to the higher total protein content in these cases for all 5 proteins studied were more common regardless of the degree of increase of the total protein content. At examination 2—3 weeks after onset the pathologic precipitates were still more common than among the convalescents. The difference in frequency between fibrinogen and α_2-macroglobulin was also still small, but β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_{ss}-globulin precipitates were again more common in the severe cases of meningoencephalitis. As before, these differences occurred irrespective of the degree of increase of the total protein content.

The longitudinal investigation of 77 patients who had been ill for 2—3 weeks and of 36 examined 6—10 weeks after onset showed that in post infectious states immunoelectrophoretic analysis can disclose disorders of the C.N.S. either by demonstrating the presence of transproteins in the C.S F as a sign of a disorder of the blood-C.S.F barrier or by the occurrence of immunoglobulin precipitates not normally occurring in the C.S F

GENERAL SUMMARY

The investigation was carried out on 131 patients at the *onset* of *virus meningoencephalitis*. Aetiological diagnoses were made in 61 cases. 68 % were classified as having a mild form of the disease. A significant correlation was found between the severity of the disease and the increase of the total protein content. The C.S.F. was examined immunoelectrophoretically regarding 3 plasma proteins normally not occurring in the C.S.F. and α_2-macroglobulin and γ_A-globulin which normally occur in low concentration. A marked α_2-macroglobulin precipitate was noted in 67 patients, β_1-lipoprotein in 50, fibrinogen in 75, γ_1-macroglobulin in 50 and marked anodic γ_A-globulin precipitate in 38. To indicate a possible difference between these 5 proteins the first 3 are called transproteins and the last 2 immunoglobulins. A marked anodic γ_A-globulin precipitate was noted more often in adults and a marked α_2-macroglobulin more often in children. β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_A-globulin were more common in the severe cases. All 5 proteins studied, with the exception of marked anodic γ_A-globulin, were demonstrated more often among patients with a high cell content. β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_A-globulin precipitates were demonstrated more frequently in patients with a high total protein content. As many as 77 % of those with a normal total protein content showed an abnormal protein pattern.

C.S.F. and serum from 77 persons were analysed *2—3 weeks after onset* of disease. 53 persons were convalescent. The cell count was normal in 19 and the total protein content in 38. A highly significant correlation was found between the severity of the disease and the total protein content. A marked α_2-macroglobulin precipitate was found in 19, β_1-lipoprotein in 8, fibrinogen in 14, γ_1-macroglobulin in 29 and marked anodic γ_A-globulin precipitate in 17. Immunoglobulin precipitates were found more often in adults. β_1-lipoprotein, γ_1-macroglobulin and marked anodic γ_A- globulin precipitates were statistically more common among those who were still ill. A highly significant correlation was found between the total protein content and the number of precipitates demonstrated. In [illegible] % of the patients with a normal total protein content the protein pattern

was, however abnormal. γ_1-macroglobulin and marked anodic γ_{ss}-globulin precipitates occurred for the first time at this examination more often than any of the other proteins. In addition β_1-lipoprotein och fibrinogen had disappeared to a much larger extent than γ_1-macroglobulin. This explains why γ_1-macroglobulin was the commonest finding in this phase of the disease.

6—10 weeks after onset C.S.F and serum were analysed from 20 healthy patients who had recovered and from 16 who were still ill. There was a tendency to a higher cell count and a higher total protein content in the latter group Of the former group 15 had a normal protein pattern in C.S.F and of 16 still ill, 10 had an abnormal protein pattern. γ_1-macroglobulin was the most common finding Immunoglobulin precipitates were found as the only pathologic findings even in C.S.F with normal cell and total protein content. In no case did a precipitate occur for the first time in this phase of the disease.

In 27 patients with *bacterial meningitis* the C.S.F and serum were studied on various occasions. Twenty-four of the patients were examined in the acute stage of the disease. Marked α_2-macroglobulin precipitates were found in 88 % β_1-lipoprotein in 76 % fibrinogen in 80 % γ macroglobulin in 52 % and marked anodic γ_{ss}-globulin precipitate in 72 % of the cases. Immunoglobulin precipitates were found more often in adults than in children. Therapy was as a rule accompanied by a tendency to relatively rapid improvement of the protein pattern. Of 13 patients re-examined within 2—6 days the pattern was found to be normal in 3. After 10—20 days all together 10 of 18 patients showed a normal protein pattern. Of the 8 with an abnormal protein pattern, 6 showed an atypical precipitate with antifibrinogen serum The protein pattern often became normal before the total protein content. Later in the course of the disease 3 patients had immunoglobulin precipitates which had not been demonstrable during the last preceding weeks. γ_1-macroglobulin was noted in 1 case 4 months after onset and marked anodic γ_{ss}-globulin precipitate after 7 months.

The observations set forth above appear to warrant the following conclusions

1 In infectious diseases of the C.N.S the clinical course is largely reflected by changes in the immunoelectrophoretic pattern of the C.S.F

2 Such changes occur even when the total protein content of the C.S.F is normal.

3 In these diseases immunoglobulins often occur and sometimes persist as the only immunoelectrophoretic abnormality in the C.S.F even after clinical recovery This investigation lends strong support to the assumption that in these infections immunoglobulins are formed intrathecally

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DENCKER, S. J., BÄCKERSTAM, R. and SWAHN, B.: Mikroimmunoelektrofores, ett bidrag till liquordiagnostiken. Nord. Med. 64 477 1960.

DENCKER, S. J., BÄCKERSTAM, R. and SWAHN, B. Demonstration of large blood proteins in cerebrospinal fluid. Neurol. 11 441 1961

DENCKER, S. J. and SWAHN, B. Identification of separate proteins in cerebrospinal fluid with the aid of microimmuno-electrophoresis. Acta psychiat. neurol. scand. 36, Suppl. 150: 3 9, 1961

DENCKER, S. J. and SWAHN, B. Clinical value of protein analysis in cerebrospinal fluid. Lunds Universitets Årsskrift N.F avd. 2, Bd 57 No 0, 1961 a.

✓ DENCKER, S. J. SWAHN, B. and UHRBRAND, B. Protein pattern of cerebrospinal fluid during the course of acute polyradiculoneuropathy Acta med. scand. 175: 499, 1964.

ENDERS, J. F. Mumps. Viral and Rickettsial Infections of Man, Philadelfia, 1959.

FANCONI, G Die Poliomyelitis und ihre Grenzgebiete, Schwabe, Basel, 1945.

FINK, C. S., MILLER, W. E., DORWARD, B. and LOSPALLUTO, J.: The formation of macroglobulin antibodies. J clin. Invest. 4 412, 962.

FLACH, A. Fall av andningsförlamning vid poliomyelit, behandlade i respirator. Nord. Med. 11: 422, 194

FRICK, E. Immunophoretische Untersuchungen am Liquor cerebrospinalis. Klin. Wschr 37 645, 959.

FRICK, E. Quantitative Bestimmung des Transferrins im normalen und pathologischen Liquor cerebrospinalis. Klin. Wschr 4 75, 963.

FRICK, E. and SCHEID-SEYDEL, L. Immunologische Untersuchungen an Liquoreiweisskörpern. Z. ges. exp. Med. 29 22 957

FRICK, E. and SCHEID-SEYDEL, L. Untersuchungen mit J[131] markiertem Albumin über Austauschvorgänge zwischen Plasma und Liquor cerebrospinalis. Klin. Wschr 36: 66, 1958.

FRICK, E. and SCHEID-SEYDEL, L. Untersuchungen mit J[131] markiertem γ-globulin zur Frage der Abstammung der Liquoreiweisskörper Klin. Wschr 36 857 958 a.

GAVRILESCO, K., COURCON, J HILLION, P URIEL, J LEWIN, J and GRABAR, P Étude du liquide céphalo-rachidien, humain normal par la méthode immuno-électrophorétique. Bull. Soc. Chim. Biol. 37 803, 955.

GORDON, A., KEIL, B. and SEBESTA, K. Electrophoresis of proteins in agar jelly Nature 64: 498—499, 1949.

GRABAR, P Immunochemical methods in studies on proteins. Advances in Protein chemistry XIII 958.

GRABAR, P Immunoelectrophoretic analysis. Immunological methods. Symposium. Blackwell Sc. Publ., Oxford, 964.

GRABAR, P and WILLIAMS, C. A. Jr Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines. Application sérum sanguin. Biochim. biophys. Acta 93, 1953.

HAYMAKER, W LEWIS, K. R. and SCHWARTZ, E. Pathology of the meningo-encephalomyelitides due to viruses, rickettsiae, trypanosomes, toxoplasma and fungi. Huit coll. de biol. clin. Presses Académiques Européennes, 958.

HEREMANS, J F Les globulines sériques du système gamma. Masson et C[ie] Paris, 960.

HEREMANS, J F Die Immunoelektrophorese und ihre klinische Bedeutung. Ergebn. inn. Med. Kinderheilk. 10 169, 963.

HIRSCHFELD, J Characterization of precipitating components in normal human sera obtained by an immuno electrophoretic technique. Acta path. microbiol. scand. 49 255, 960.

References

ADAIR, C. V., GAUL, R. L. and SMADEL, J. E. Aseptic meningitis. A disease of diverse etiology *Ann. int. Med. 39* 675 1953

BANG, H. O and BANG, J Involvement of the central nervous system in mumps. Acta med. scand. *113* 487 1943

BAUER, H. Die Cerebrospinalflüssigkeit. *Der Internist 1* 85 1961

BEDJANIC, M., RUS, S., KMET J and VESENJAK-ZMIJANAK, J Virus meningo-encephalitis in Slovenia. Bull. Org. mon. Santé *12* 503, 1955

BOWSHER, D Cerebrospinal fluid *dynamics in health and disease. Thomas, Springfield Ill.,* 1960.

BREWIS, E. G Polio as a cause of meningitis and meningo-encephalitis. Virus Meningo-Encephalitis. Ciba Foundation N o 7 Churchill, 1961

BURRELL, R. G RHEINS, M. and BIRKELAND, J M. Tuberculous antibodies demonstrated by agar diffusion. Amer Rev Tuberc. Pulm. Dis. *74* 239, 1956.

BURTIN P Études sur les protéines du liquide céphalo-rachidien. Clin. chim. acta *4* 72, 1959

BURTIN P Les protéines du plasma humain normal. In Grabar P and Burtin, P Analyse immuno-électrophorétique. Masson et C^ie^ Paris 1960.

BURTIN P Les protéines du liquide céphalo-rachidien. In Grabar P and Burtin, P Analyse immuno-électrophorétique Masson et C^ie^ Paris 1960.

BURTIN, P and PORCIDALO, J J Études quantitative des gamma-globulines dans le liquide céphalo-rachidien par une méthode immunochemique. Presse méd. *62* 1072 1954.

CARPENTER, R. R. and PETERSDORF R. G The clinical spectrum of bacterial meningitis. Amer J Med. *33* 262 1962

CHANG, TE WEN *and* WEINSTEIN L. *Relationship of cerebrospinal fluid pleocytosis to recovery* of echo 9 virus. J Amer med. Ass. *181* 1040, 1962

CLAUSEN J The serum proteins in normal and pathological spinal fluids. Acta psych. scand. 35, Suppl. *148* 11 1960.

CLAUSEN, J Immunoelectrophoretic investigations of normal and pathologic cerebrospinal fluids. Their correlation with findings in human serum. Wld. neurol. *1* 479, 1960 a.

CLAUSEN J Some recent methods fo fractionation of proteins employed in diagnostic examination of cerebrospinal fluid. Dan. med. Bull. *9* 1 1962.

CLAUSEN, J DENCKER, S. J and SVENNERHOLM, L. Proposed standardization of analysis of cerebrospinal fluid proteins. Acta neurol. scand. Suppl 10, *40* 89, 1964.

CLAUSEN, J KROGSGAARD, A. R. and QUAADE, F Immunoelectrophoretic studies of the cerebrospinal fluid and serum in acute, mainly infectious diseases of the central nervous system. J infect. Dis. *111* 128, 1962.

CROWLE, A. J Immunodiffusion. *Acad. Press, New York, 1961*

DENCKER, S. J Personal communication, 1963.

Ouchterlony, O. Diffusion-in-gel methods for immunological analysis. Progr. Allergy 5 1 1958.

Pette, H.: Die virus meningitis. Dtsch. Z. Nervenheilk. 171: 261 1954.

Porter, R. R.: Chemical structure of γ-globulin and antibodies. Brit. med. Bull. 19 197 1963.

Quaade, F and Kristensen, K. P Meningitis purulenta. Ugeskr. Laeg. 123 1341 1961.

Rieser, S.: Cerebrospinalvaeskens dannelse og absorption. Nord. Med. 69 806, 1963.

Rosenthal, F D and Soothill, J F An immunochemical study of the proteins in cerebrospinal fluid. J Neurol. Neurosurg. Psychiat. 25: 177 962.

Scheidegger, J J Une micro-méthode de l'immuno-électrophorèse. Int. Arch. Allergy 7 103, 1955.

Scheiffarth, F Götz, H., Berg, G and Hoffmeister, H.: Immunoelectrophoretische Studien am normalen und pathologischen Liquor Klin. Wschr. 36 678, 958.

Schmidt R. M. Zur Liquordiagnostik der sogenannten aseptischen Meningoencephalitis und Panencephalitis. Dtsch. Gesundh. Wes 15 2317 1960.

Schultze, H. E. Über Glykoproteine. Dtsch. med. Wschr 83 1742, 1958.

Schultze, H. E. and Schwick, G. Immunchemischer Nachweis von Proteinveränderungen unter besonderer Berücksichtigung fermentativer Einwirkungen auf Glyko- u. Lipoproteine. Immunoelektrophoretische Studien. Behringwerk Mitteilungen 33 1 1957

Schönenberg, H. Der Liquor cerebrospinalis im Kindesalter Thieme, Stuttgart, 1960.

Smith, E. G. Purulent meningitis in infants and children. J Pediat. 45 425, 1954.

Smith, R. T and Eitzman, D. V The development of immune response. Pediatrics 33 63, 964.

Ström, J Meningitis serosa. Nord. Med. 55 857 956.

Svedmyr, A., v Zeipel, G Holmgren, B. and Lindahl, J Tick borne meningoencephalitis in Sweden. Arch. ges. Virusforsch. 8 565, 1958

Svennerholm, E., Dencker, S. J and Swahn, B. Immunoelectrophoretic studies of cerebrospinal fluid Investigations in patients with tumor in the central nervous system. Neurol. 989, 96

Tobler, R. Frühkindliche transitorische Agammaglobulinämie. Helv paediat. Acta 13 339, 958.

Uriel, J and Grabar, P Étude des lipoprotéines sériques par l'électrophorèse en gélose et l'analyse immunoélectrophorétique. Bull. Soc. Chim. biol. 38 1253, 1956.

Unberg, B., Dencker, S. J and Swahn, B. Protein pattern of cerebrospinal fluid in meningitis and meningo-encephalitis. Acta med. scand. 71 7 5, 962

Waddell, W J A simple ultraviolet spectrophotometric method for the determination of protein. J Lab. clin. Med 48 3 1 958.

Wehrle, H. A. The benign aseptic meningitis. Med. Clin. North. Amer 43 1451 1959.

Wickman, J Beiträge zur Kenntnis der Heine-Medinschen Krankheit. Karger, Berlin, 1907

Widell, S. On the cerebrospinal fluid in normal children and in patients with acute abacterial meningoencephalitis. Acta paediat. Suppl. 1,8, 1958.

Wiedermann, G., Reinhardt F and Kunz, Ch. Antikörperbildung bei Frühsommer Meningoencephalitis. Zbl. Bakt., I. Abt. Orig. 89 15, 963.

Wieme, R. J Méthode nouvelle permettant la démonstration directe de macroglobulines. Rev belge Path. 26 62, 957

Wieme, R. J. Studies on agargel electrophoresis. Arscia Uitgaven, Bruxelles, 1959.

Williams, C. A. and Grabar, P Immunoelectrophoretic studies on serum proteins. J Immunol. 74 58, 955.

HITZIG, W H. Zur quantitativen Bestimmung spezifische Proteinfraktionen. Int. Arch. Allergy *19* 145 1961
HITZIG, W H. *Die Plasmaproteine in der klinischen Medizin.* Springer Berlin, 1963
HOLMGREN B LINDAHL, J v ZEIPEL, G and SVEDMYR, A. Tickborne meningoencephalitis in Sweden. Acta med. scand. *164* 507 1959
HUMPHREY J H. and WHITE, R. G Immunology for students of medicin. Blackwell, Oxford, 1963
KAHLMETER, O. Clinical aspects of ECHO-viruses. Virus Meningo-Encephalitis. Ciba Foundation No. 7 Churchill, 1961
KILHAM, L. Mumps meningoencephalitis with and without parotitis. Amer J Dis. Child. *78* 324, 1949
KORNGOLD L. and LEEUWEN, G The effect of the antigen s molecular weight on the curvature of the precipitin line in the Ouchterlony technique. J Immunol. *78* 172, 1957
LATERRE, E. C., HEREMANS, J F and DEMANET G La pathologie des protéines du liquide céphalo-rachidien. Étude électrophorétique et immuno-électrophorétique. Rev neurol. *107* 500, 1962
LAURELL, C. B LAURELL, S. and SKOOG, N Buffer composition in paper electrophoresis. Clin. Chim. *2* 99, 1956.
LEPOW M. L., COYNE, N THOMSON L. B., CARVER, D H. and ROBBINS, F C. A clinical epidemiological and laboratory investigation of aseptic meningitis during the four year period, 1955—1958. New Engl. J Med. *266* 1188, 1962
LOSPALLUTO, J MILLER, W., DORWARD, B. and FINK, C. W The formation of macroglobulin antibodies. J Clin. Invest. *41* 1415, 1962.
MACRAE, A. D Viruses as a cause of meningo-encephalitis. Virus Meningo-Encephalitis. *Ciba Foundation Group 7 Churchill, 1961*
MACPHERSON, C. F C. and COSGROVE, J B. R. Immunochemical evidence for a gamma globulin peculiar to cerebrospinal fluid. Canad. J Biochem. Physiol. *39* 1567 1961
MATTERN, P A propos de la présence de $\beta_2$macroglobuline dans le liquide céphalo-rachidien. C. R. Soc. Biol. *156* 158, 1962.
MATIAR, H. and SCHMIDT C. Die Veränderungen der Liquorproteine bei entzündlichen Erkrankungen der Meningen. Dtsch. Z. Nervenheilk. *176* 200, 1957
MEYER, H M., JOHNSON R. T., CRAWFORD, I P DASCOMB, H. E. and ROGERS, N G Central nervous system syndromes of "viral etiology Amer J Med. *29* 334, 1960.
MIES, H. J Einengung von Liquor cerebrospinalis als Vorbereitung zur Papierelektrophorese. Klin. Wschr *31* 159, 1953.
MÜLLER, R., NYLANDER, L, LARSSON L. E., WIDÉN L. and FRANKENHAEUSER, M. Sequelae of primary aseptic meningo-encephalitis. Acta psychiat. scand. Suppl. 126, *33* 1958.
NUSSENZWEIG, V and SELIGMANN M. De la dégradation par la plasmin du fibrinogèn humain et de la *fibrine, à differéntes stades. Rev Hémat. 15* 451 1960.
v OLDERSHAUSEN H. F Klinische Beobachtungen über eine neuartige primäre aseptische Meningo-encephalitis. Dtsch. med. Wschr *82* 442, 1957
v OLDERSHAUSEN, H. F Virus *Meningo-encephalitis. Ciba Foundation, N o 7* Churchill, 1961
OUCHTERLONY O In vitro method for testing the toxin-producing capacity of diphteria bacteria. Acta path. microbiol. scand. *25* 186, 1948.
OUCHTERLONY O Antigen-antibody reactions in gels. Acta path. microbiol. scand. *32* 231 1953.

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SUPPLEMENTUM 428

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1963—1964

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SVEN E. NILSSON HENNING LINDHOLM,
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Supplementum 428

From the Department of Medicine (Head Henning Lindholm, M. D.),
the Department of Ophthalmology (Head Herman Grönvall, M. D.),
and the Department of Roentgenology (Head Nils Frostberg, M. D.),
Kristianstad General Hospital, Kristianstad

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Contents

Introduction

This investigation is concerned with a number of clinical, anthropometric and laboratory determinations in a representative sample of an adult population (20—79 years) and calculation of the normal values and ranges of variation of such measurements.

Attention was given in particular to the effect of age sex, and bodybuild on the results of the oral glucose tolerance test and the usefulness of the test in the diagnosis and in the estimation of the risk of a given person developing diabetes mellitus.

The presentation also includes a brief account of certain analyses of anthropometric measurements (including certain simple expressions of the fatfree weight and bodyfat)
blood pressure,
pulse frequency and some electrocardiographic findings
body temperature
hemoglobin and number of erythrocytes,
number and distribution of leucocytes,
amounts and distribution of various blood proteins as estimated by electrophoresis,
glutamic pyruvic transaminase
calcification of the arteries of the lower limbs,
cataract and arcus lipoides corneae and intra-ocular pressure.

It is intended to utilize the results of the investigation as reference values in a large scale study of a diabetic population.

The data were treated in an electronic computer by Torgil Ekman, *fil kand.*

The studies were carried out at the departments of internal diseases, ophthalmology and roentgenology Kristianstad general hospital, during the months of August and September 1963 and February 1964

The investigation and its publication were supported by grants from *Kristianstads läns landsting*

Material

The investigation was based mainly on a random selection of a normal population—hereinafter called series A. For some purposes values obtained in two other studies (series B and C) were also used.

Series A

This series consisted of a sample of persons who on December 31 1962 were living in Kristianstad (a town with 26 166 inhabitants) or in the surrounding parishes (Nosaby Fjälkinge Åhus, Vä Everöd Arnslöv with altogether 23,352 inhabitants). The social structure in this area in the southernmost part of Sweden is largely representative of Sweden as a whole, as is apparent from comparison of the occupational distribution of the males in the sample and in the entire population of Sweden (Table 1).

The persons belonging to this series were selected according to date of birth, all those born on January 20th respectively July 20th of the years between 1884 and 1943 making up the primary selection. When any of the one year-classes did not include at least one man and one woman from Kristianstad and one man and one woman from the surrounding parishes a man and/or woman born on April 20th or October 20th was selected. If more than one man and/or woman had been born on that date, the choice was decided by the alphabetic order of the initial of the surname.

Table 1 *Comparison between occupational distribution of invited males in series A and that of entire Swedish male population (according to Statistical Abstracts of Sweden 1963) and examined as per cent of persons invited in various occupational groups*

	Urban	Rural	Total	Sample	% Entire country	Examined	
						Number	Percentage of invited
Agriculture forestry fishing	1	19	20	14	18	16	75
Manufacturing, construction etc	35	27	62	44	50	46	74
Transport and communication	6	3	9	6	9	5	66
Commerce	19	9	28	20	10	23	89
General administration	10	7	17	12	10	14	91
Miscellaneous	2	2	4	3	3	2	50

Table 2. Survey of Series A.

		1	2	3	4	5	6	7	8	9	10
Males	60—79	54	2	4	1	47	36	77	4	0	7
	40—59	53	0	1	2	50	42	84	3	3	2
	20—39	49	0	0	6	43	31	72	1	0	11
		156	2	5	9	140	109	78	8	3	20
Females	60—79	50	5	3	0	42	28	67	8	0	6
	40—59	46	1	1	0	44	36	82	2	1	5
	20—39	49	0	1	3	45	34	76	3	2	6
		145	6	5	3	131	98	74	14	3	16
Total		301	8	10	12	271	207	76.4	22	6	36

1. Total recorded
2. With diabetes mellitus
3. With some other chronic disease
4. Moved from district
5. Total invited
6. Examined
7. Examined in per cent of all invited
8. Would not consent to examination
9. Could not attend examination (various reasons)
10. Untractable

This primary material consisted of 301 persons, all of whom were invited by letter to take part in the investigation to be carried out at Kristianstad general hospital. Those persons who did not reply or present themselves for examination were contacted again by post and, if necessary by phone or personally.

As a remuneration for loss of time etc the participants were offered 25 Swedish crowns, taxi fares to and from the hospital, and a free lunch there.

The composition of the series is given in Table 2 which also includes the reasons for non-co-operation.

Of the originally 301 persons registered 18 were excluded owing to diabetes mellitus or disabling diseases contraindicating the performance of the examination planned. The diseases noted in these 18 patients are listed below (F (female) M (male) I (insulin) T (d.m treated with tablets) D (only diet) Figures indicate present ages.)

Diabetes mellitus	8 (F 46 D F 61 T F 63 T F 64 T M 68 D M 72 I F 72 T F 75 T)
Organic heart disease with cardiac incompensation	3 (M 57 M 62 F 80)
Myocardial infarction	1 (M 67)
Arterial hypertension with cardiac involvement	1 (F 64)

The investigation was based mainly on a random selection of a normal population—hereinafter called series A. For some purposes values obtained in two other studies (series B and C) were also used.

Series A

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						Number	Percentage of invited
Agriculture, forestry fishing	1	19	20	14	18	15	75
Manufacturing, construction etc.	35	27	62	41	50	46	74
Transport and communication	6	3	9	6	9	5	56
Commerce	19	9	28	20	10	25	89
General administration	10	7	17	12	10	16	94
Miscellaneous	2	2	4	3	3	3	60

Methods

GLUCOSE TOLERANCE TEST

The patients were given an oral dose of 30 g glucose per m body surface calculated according to Dubois nomogram. The glucose content of capillary blood was measured in samples collected immediately before and 30 60 90 120 150 and 180 minutes after the dose had been given.

The blood sugar was determined by the orthotoluidine method of HULTMAN (1959) According to this method the amount of aldoses is measured, which under normal conditions is equal to the amount of "true glucose Two capillary samples were taken on each occasion to check the accuracy of the method. The difference between the two determinations was, on the average 0 9 mg/100 ml, which corresponds to 4 % of the S.D

The sugar in the urine was determined in samples collected 60 120 and 180 minutes after the dose had been given. Benedict's quantitative test was used.

The subjects were requested to present themselves in the fasting state and without having smoked on that day before the examination, which was done between 7.30 and 10 30 a.m. It was not considered necessary to prescribe any dietary measures because all of the subjects examined were regarded as adequately nourished (WILKERSON et al. 1960) To avoid physical exertion, all patients were requested to come to the hospital by taxi-car During the examination the patients were instructed to rest on a bed.

No difference in glucose level was found between the tests performed in August—September and those performed in February

All sampling and laboratory work were done by two well trained assistants.

ANTHROPOMETRIC VALUES

All persons were weighed in light underclothes, for which an allowance of 0.5 kg was made.

In the analysis of the bodybuild the following factors were determined

Skeletal length factor expressed as bodyheight

Skeletal sturdiness factor expressed as femoral condylar breadth and bistyloid radio-ulnar breadth, measured

Status after cerebral haemorrhage	2 (F 53 M 73)
Disseminated sclerosis	1 (M 43)
Senility	1 (F 78)
Uterine cancer	1 (F 65)

Twelve persons had moved from the district.

Table 3 Distribution of previous diseases in patients examined

Disease	Males	Females	Total
Cerebral hemorrhage	1	0	1
Myocardial infarction	2	0	2
Peptic ulcer	6	5	11
Cholecystopathy	6	18	24
Carcinoma	1	1	2
Tuberculosis	2	2	4
Bronchial asthma	4	2	6

The observed incidence of other existing or previous diseases among the 207 persons examined, which corresponded to that expected (NILSSON 1964) is given in Table 3 The diseases were considered to have no substantial effect of the general condition of the persons at the time of the examination

Those persons who did not co-operate included a relatively large group of elderly women. It might also be mentioned that of the urban population, 90 % of the males and 80 % of the females co-operated against 67 % and 70 % of the rural population. Only 51 % (20/39) of the unmarried males and 60 % (28/47) of the unmarried females invited presented themselves for examination. The groups from the town and the rural district, married and unmarried and examined in August—September respectively in February were not quite comparable and were therefore generally not studied for any differences.

Series B

This series consisted of 132 consecutive cases of myocardial infarction seen at the department of internal diseases, Malmö general hospital in 1961 and 1962 and is described elsewhere (SIEVERS 1963)

Series C

This series was made up of 471 males, aged 18 years and belonging to the 6th military registration district and inducted in the autumn of 1959 The 6th military registration district covers the northern part of Scania including Kristianstad and has 430 000 inhabitants. The subjects were selected either because they had a close relative with diabetes or because their registration number immediately followed that of a relative of a diabetic.

The series has been described in detail elsewhere (NILSSON 1962 1964)

taken of the thigh. The frontal view of the lower leg was taken with the leg turned slightly inward to enable estimation of the space between the tibia and the fibula while the lateral view was taken with the leg in the normal position. The popliteal region was inspected either in a lateral view taken over the thigh or in a lateral view of the lower leg. The foot arteries were judged in frontal and oblique views taken mainly over the area of the metatarsal bones and areas around the malleoli.

The *intraocular pressure* was measured with a Schiötz x-tonometer. One and the same instrument was used throughout and the 0 position of the tonometer was checked every day. The measurements were made as far as possible at the same time of the day. The subjects were examined in the supine position.

The degree of *arcus lipoides corneae* (a.l.c.) was estimated by examination with a corneal microscope.

The *degree of cataract* was estimated with the aid of a corneal microscope, and in transmitted light with an ophthalmoscope.

STATISTICAL METHODS

Arithmetic means, standard deviations (S.D.) and *coefficients of correlation* (r) were calculated according to standard statistical methods.

In the evaluation of correlation coefficients the concordance and structure of the correlations must be taken into account. Therefore no degrees of significance are given in the tables. In the evaluation of the probability of a single correlation to differ from zero the *following criteria of significance* for different pairs of observations were used in the present investigation.

	Pairs of observations	Level of significance 0.1	0.05	0.01	0.001
Subgroups males, females	25	0.27	0.32	0.42	0.52
Total males, females	100	0.16	0.19	0.25	0.32
Total	200	0.12	0.14	0.18	0.23

according to MARTIN (1928) and LINDEGÅRD (1953 1956) This sturdiness factor is highly correlated with muscle mass (LINDEGÅRD 1953 1956)

Fat free bodyweight calculated according to v DÖBELN (1959 1961) and according to the formula $FFW_I = 15.1 (H^2 F R 100)^{0.712}$ as well as the formula $FFW_{II} = 1.2 + 162 H^2 R$ (FFW is fatfree weight, H height, F sum of right and left femoral condylar breadths and R sum of right and left radio ulnar bistyloid breadths FFW is given in kilograms and H F and R in metres)

Weight of bodyfat assessed by the difference between total bodyweight and fatfree bodyweight, calculated according to v DÖBELN As a rule, body fat has been expressed as per cent of total bodyweight This quotient is highly correlated with skinfold measurements as had been shown previously in Series C (NILSSON 1962)

OTHER EXAMINATIONS

The *blood pressure* was measured after the subject had been lying for 5 minutes, immediately after he had stood up and again after he had been standing for 3 minutes. For this purpose a mercury manometer of type ERKA was used. The diastolic pressure was registered as the pressure at which the sounds disappeared

Body temperature was registered by insertion of the thermometer into the rectum for 3 minutes after the patient had been resting

Erythrocyte sedimentation rate was measured according to WESTERGREN (1924)

Hemoglobin was determined spectrophotometrically after dilution with 0 04 % ammonia. The error of the method is less than 2 %

The *erythrocyte count* was determined in a celluscope with a calculated error of the method of less than 0.2 mill/mm

The *leucocyte count* and differential leucocyte count (polynuclear and mononuclear cells) were made with the use of a Burker counting chamber

The *serum glutamic pyruvic transaminase* was determined according to REITMAN & FRANKEL (1957) and Sigma (1960)

Electrophoresis was done with an LKB apparatus, a buffer solution according to LAURELL et al (1958) and staining with 0 5 % bromphenol blue

E C G The following leads were used I II III, CR AVR AVL AVF and V_{1-6}

Roentgenography of lower limbs Radiographs were taken of the soft tissue with a focus film distance of about 100 cm., primary diaphragm and Potter Bucky diaphragm Two views, one at right angles to the other were

taken of the thigh. The frontal view of the lower leg was taken with the leg turned slightly inward to enable estimation of the space between the tibia and the fibula while the lateral view was taken with the leg in the normal position. The popliteal region was inspected either in a lateral view taken over the thigh or in a lateral view of the lower leg. The foot arteries were judged in frontal and oblique views taken mainly over the area of the metatarsal bones and areas around the malleoli.

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Total males, females	100	0.16	0.19	0.25	0.32
Total	200	0.12	0.14	0.18	0.23

The influence of sex and age on anthropometric measurements is apparent from Tables 4 and 5 from which it is clear that fatfree weight (FFW) measured according to v. DÖBELN (1959, 1961) remained fairly constant between 20 and 79 years of age in males as well as in females.

On comparison of fatfree weight (FFW) measured according to formula $FFW = 15.1\ (H^2\ F\ R)^{0.712}$ and according to formula $FFW = 1.2 + 102\ H^2\ R$ the latter gave lower average values. The standard deviation found with the latter formula was somewhat larger for all age groups.

The difference between the total weight and fatfree weight was taken as a measure of bodyfat. The values obtained agreed with those reported by BROŽEK et al. (1953) and YOUNG et al. (1963) who used the densitometric technique, but were lower throughout than the values obtained in isotope studies by ANDERSON (1963) and MENEELY et al. (1963) (Table 6). The

Table 4 *Anthropometric findings in series A and C*

		Series A 20—39 years			Series A 40—59 years			Series A 60—79 years			Series C 18 years	
Males	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
Weight	31	71.9	9.4	42	76.2	9.5	36	76.6	10.3	467	64.3	7.5
Height	31	175.4	6.4	42	173.1	5.0	36	171.4	4.6	467	175.6	6.5
Condyl. breadth	31	9.60	0.39	42	9.56	0.37	36	9.64	0.36	205	9.56	0.42
Bistyl. breadth	31	5.87	0.26	42	6.00	0.26	36	5.94	0.29	205	5.82	0.30
Fatfree weight	31	59.7	4.9	42	59.2	4.6	36	58.4	4.5	205	59.1	5.8
Per cent bodyfat	31	16.1	8.4	42	21.3	10.8	36	22.5	9.6	205	11.6	9.1
Females	n	mean	SD	n	mean	SD	n	mean	SD			
Weight	34	64.3	10.6	37	67.1	11.9	28	70.5	11.6			
Height	34	163.4	5.0	37	161.8	5.5	28	162.0	6.7			
Condyl. breadth	34	8.81	0.39	37	8.73	0.38	28	9.01	0.61			
Bistyl. breadth	34	5.15	0.29	37	5.16	0.23	28	5.24	0.31			
Fatfree weight	34	46.0	4.3	37	45.1	4.0	28	47.0	4.2			
Per cent bodyfat	34	27.5	9.3	37	30.8	10.6	28	32.8	9.9			

Table 5. *Correlation between age and various anthropometric findings.*

	Weight	Height	Condylar breadth	Fatfree weight	Bodyfat	Body surface
Total	.18	— .13	.13	.03	.18	.08
Males	.18	— .23	.01	— .13	.24	— .02
Females	.19	— .13	.20	.09	.18	.13
M 20—39	.16	— .26	— .05	.00	.12	.05
40—59	— .11	— .26	— .26	— .25	.13	— .22
60—79	— .12	— .19	.33	— .01	— .16	— .16
F 20—39	.40	.20	.25	.32	.29	.40
40—59	— .08	— .07	— .01	— .05	— .03	.08
60—79	— .27	— .44	.13	— .13	— .32	— .40

difference, which was larger for young men and elderly women may have been due to differences in the composition of materials.

It is clear from Table 7 that in both series bodyweight is more dependent on the amount of bodyfat than on fat free weight. Correlation coefficients of the same numerical order have been observed by BARTER & FORBES (1963) for example. A close correlation was found between stature and skeletal sturdiness expressed as condylar breadth respectively radioulnar bi styloid breadth.

Table 6. *Body fat in per cent f total body weight in age-classed mal s and females in seri A and B as well as according t Bro ek et al (1953) a Döbeln (1956), Young t al (1963) and And rson (1963).*

	Age	Series A	Series B	Brozek	Döbeln	Young	Andersson
Males	20—29	13.1	12.9	12.1	10.6		19.1
	30—39	18.8	16.4	17.3			21.4
	40—49	22.6	20.6	21.8		21.3	21.6
	50—59	21.3	19.6	23.9			21.6
	60—69	21.0	21.2			20.5	26.8
	70—79	19.6	20.1				21.9
Females	20—29	22.2	22.8	28.5	20.3	28.7	33.8
	30—39	20.4	21.8	30.8		28.5	32.0
	40—49	28.7	31.1	31.2		33.3	36.5
	50—59	20.9	31.6	38.5		41.9	38.0
	60—69	33.2	32.0			44.6	40.3
	70—79	28.3	28.7				

The influence of sex and age on anthropometric measurements is apparent from Tables 4 and 5 from which it is clear that fatfree weight (FFW) measured according to v DÖBELN (1959 1961) remained fairly constant between 20 and 79 years of age in males as well as in females

On comparison of fatfree weight (FFW) measured according to formula FFW = 15 1 $(H^2 F R)^{0.712}$ and according to formula FFW = 1.2 + 162 H^2 R the latter gave lower average values The standard deviation found with the latter formula was somewhat larger for all age groups

The difference between the total weight and fatfree weight was taken as a measure of bodyfat The values obtained agreed with those reported by BROZEK et al (1953) and YOUNG et al (1963) who used the densitometric technique but were lower throughout than the values obtained in isotope studies by ANDERSON (1963) and MENEELY et al (1963) (Table 6) The

Table 4 *Anthropometric findings in series A and C*

		Series A 20—39 years			Series A 40—59 years			Series A 60—79 years			Series C 18 years	
Males		mean	SD	n	mean	SD		mean	SD		mea	SD
Weight	31	71 9	9 4	42	76.2	9.5	36	76 6	10.3	467	61.3	7.5
Height	31	175.4	6.4	42	173.1	5.0	36	171 4	4 6	467	173.6	6 5
Condyl breadth	31	9 60	0.39	42	9.56	0.37	36	9 64	0.36	203	9.56	0 42
Bistyl breadth	31	5.87	0.26	42	6.00	0.26	36	5 94	0.29	208	5.82	0.30
Fatfree weight	31	59 7	4 9	42	59.2	4.8	36	58.4	4.5	206	59 1	5 6
Per cent bodyfat	31	16.1	8.4	42	21.3	10.8	36	22.5	9 8	205	11 6	9 1
Females	n	mea	SD		mea	SD	n	mean	SD			
Weight	34	64.3	10 6	37	67 1	11.9	25	70.5	11 6			
Height	34	163 4	5.0	37	161.6	5.5	25	162.0	6.7			
Condyl breadth	34	8.81	0.39	37	8.73	0.38	25	9 04	0.61			
Bistyl. breadth	34	5 15	0.29	37	5.16	0.23	25	5.24	0 31			
Fatfree weight	34	46 0	4 3	37	45 1	4 0	25	47.0	4.3			
Per cent bodyfat	34	27.6	9.5	37	30.8	10 6	25	32.8	9.0			

The curve for glucose in the blood after oral administration of glucose can be described by

A Fasting blood sugar level
B. Rate of absorption
C. Highest level after administration of the dose
D Duration of increase
E. Declination of the curve

It may be assumed that all of these 5 factors are influenced by sex, age, and body build, all of which must therefore be taken into account in the interpretation of the curve

The correlation between fasting blood sugar level and glucose level at different intervals after the ingestion of glucose is apparent from Table 8,

Table 8. *Coefficients of correlation between blood sugar level fasting and at different intervals after administration of glucose.*

	0	30'	60'	90'	120'	150'	180'
0		0.52	0.56	0.57	0.55	0.57	0.50
30'	0.52		0.72	0.42	0.24	0.24	0.15
60'	0.56	0.72		0.76	0.53	0.40	0.16
90'	0.57	0.42	0.76		0.81	0.65	0.28
120'	0.55	0.24	0.53	0.81		0.82	0.57
150'	0.57	0.24	0.40	0.65	0.82		0.83
180'	0.50	0.15	0.16	0.28	0.57	0.83	

from which it is clear that the fasting blood sugar level tends to partly determine the level of the entire curve.

The influence of glucose excreted in the urine can be neglected in an investigation of the shape of the blood sugar curve following ingestion of about 50 g glucose.

Table 9 *Means and standard deviations of blood sugar (mg/100 ml) in fasting stat and at different intervals after administration of glucose*

		Fasting		30'		60'		90'		120'		150'		180'	
Sex	Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males	20—39	92.5	8.3	149.5	30.1	107.6	19.6	91.0	17.3	79.6	11.6	78.2	10.3	78.7	10.8
	40—59	96.9	10.7	165.2	37.7	142.6	23.9	111.2	26.3	88.4	20.4	76.4	13.2	78.0	9.5
	60—79	99.7	11.9	164.0	26.6	164.9	30.6	132.8	27.1	102.6	32.0	84.4	22.4	78.9	16.8
Females	20—39	91.6	7.8	147.6	22.6	113.6	21.4	100.5	15.0	88.5	17.0	78.5	11.7	73.9	10.2
	40—59	93.9	12.4	180.8	28.9	123.1	29.4	129.2	23.7	98.8	22.7	78.3	13.9	76.1	11.7
	60—79	98.4	13.6	167.9	37.6	150.7	37.4	128.2	41.3	106.4	34.7	87.5	29.0	78.9	16.3

Table 7 *Correlation between various anthropometric findings.*

		Height	Condylar breadth	Bistyloid breadth	Fatfree weight	Total bodyfat	Relat. bodyfat
Weight	Total	41	62	48	.52	73	.53
	M	19	57	.30	43 40**	.57 77**	.81
	F	30	.56	40	.50 .50*	92 76**	.82
	M 20—39	.50 .58	63 47*	.37 65*	61 67*	.84	78 49*
Height			61	69	88	— .23	— 43
			.38	.25	78 62	— 19 — 03**	— .26
			19	.30	74 .33	01 18 *	— 11
			64 60	*.34 40*	*.85 85**	*10*	*.03 — .24*
Condylar breadth				73	.82	06	— 14
				.37	.69	.25	18
				.53	65	.36	.25
				.38 61*	80 .82*	28	19 — .09
Bistyloid breadth					90	— 17	— .39
					66	— 02	— 12
					74	13	02
					69 75	— 03	— 11 — .29
Fatfree weight						— 18	— 41
						— 04	— 15
						15	— .03
						15	05 — .28*
Total bodyfat							.93
							93
							93
							95

Correlation coefficient series C (males aged 18 years)
Correlation coefficient according to BARTER & FORBES (1953)

The curve for glucose in the blood after oral administration of glucose can be described by

A. Fasting blood sugar level
B. Rate of absorption
C. Highest level after administration of the dose
D. Duration of increase
E. Declination of the curve

It may be assumed that all of these 5 factors are influenced by sex, age and body build, all of which must therefore be taken into account in the interpretation of the curve.

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Table 8 *Coefficient of co relati n between blood sugar level fasting and at diff rent int rvals after administration of glucose*

	0	30′	60′	90′	120′	150′	180′
0		0.52	0.56	0.57	0.55	0.57	0.50
30′	0.52		0.72	0.42	0.31	0.24	0.15
60′	0.56	0.72		0.75	0.53	0.40	0.16
90′	0.57	0.42	0.75		0.81	0.65	0.38
120′	0.56	0.34	0.55	0.81		0.82	0.57
150′	0.57	0.24	0.40	0.65	0.82		0.82
180′	0.50	0.15	0.16	0.34	0.57	0.82	

from which it is clear that the fasting blood sugar level tends to partly determine the level of the entire curve.

The influence of glucose excreted in the urine can be neglected in an investigation of the shape of the blood sugar curve following ingestion of about 50 g glucose.

Table 9 *Means and standard deviations f blood sugar (mg/100 ml) in fasting state and at diff rent interval fter administration f glucose*

		Fasting		30′		60′		90′		120′		150′		180′	
Sex	Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males	20—39	92.3	8.3	149.3	26.4	107.6	19.5	94.0	17.3	79.2	11.6	78.2	10.2	78.7	10.8
	40—59	96.9	10.7	163.2	17.7	147.6	28.9	111.7	26.3	88.4	20.4	76.4	13.2	73.0	9.5
	60—79	99.7	11.9	166.9	26.6	156.3	39.6	132.8	37.1	102.8	32.0	84.4	22.4	76.9	16.5
Females	20—39	91.3	7.8	142.6	23.5	113.8	21.4	100.9	18.0	65.5	17.0	76.6	11.7	73.9	10.1
	40—59	93.9	12.4	150.8	20.9	128.1	28.4	120.3	23.7	93.8	22.7	78.3	13.9	78.1	11.7
	60—79	98.4	13.6	162.9	27.8	149.7	37.4	138.2	41.6	106.4	34.7	87.6	25.0	78.9	16.2

Table 10 *Correlation between various anthropometric factors and blood sugar level fasting and at various intervals after glucose administration.*

Blood sugar		Weight	Height	Condyl. breadth	Fatfree weight	Bodyfat	Age
0	Total	.29	— 01	.14	09	.20	.25
	M	.32	— 06	03	— 02	40	.37
	F	.23	— 15	17	05	14	.37
30	Total	.25	.12	.20	17	07	.31
	M	17	— 06	01	— 05	.22	.39
	F	.23	01	16	.12	13	.33
60	Total	25	— .02	11	05	18	.50
	M	17	— .26	— .09	— .23	.32	.43
	F	30	— 01	19	13	17	43
90	Total	18	— 17	— 01	— 11	.37	47
	M	10	— .30	— 13	— .26	31	46
	F	.34	— 04	21	16	.21	49
120'	Total	13	— 18	— .04	— 12	.23	.39
	M	16	— 19	— 03	— 16	.30	.34
	F	20	— 08	18	10	11	39
150'	Total	15	— 10	04	— 00	11	17
	M	.32	— 04	01	— 00	.25	11
	F	12	— .20	10	.03	.03	.23
180'	Total	10	— .04	05	0.4	03	.00
	M	20	10	07	09	12	— 07
	F	03	— .20	0	00	— 03	09

EFFECT OF AGE AND SEX

The shape of the curve was found to vary substantially with age (Table 9) The values obtained agree well with those reported by previous workers (MOSENTHAL & BARRY 1950 UNGER 1957 GOTTFRIED et al 1961) — in series allowing comparison with regard to selection and age

The effect of age was most marked on values noted 60 to 90 minutes after the ingestion of glucose but it was also marked on fasting blood sugar level. No substantial difference was found with sex.

EFFECT OF BODYBUILD

The effect of bodybuild on the shape of the glucose curve is apparent from Table 10 and Fig 1 which show that the level of the glucose curve is influenced by bodyweight and then particularly by the relative amount of bodyfat. The influence was more marked for men than for women and the largest differences were noted at 60—120 minutes

Since the relative amount of bodyfat increases in males and in females up to 60 years of age (Table 6) with an accompanying tendency to higher

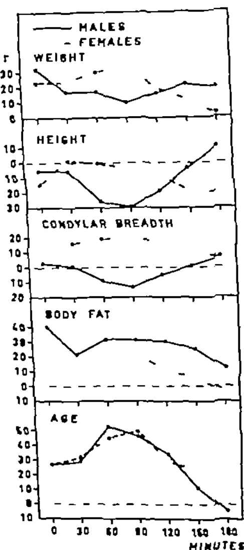


Fig. 1 Variation in shape of glucose curve with various anthropometric factors (r dif ferent inters I during glucose tolerance test.

blood sugar levels one might suspect the effect of age on the blood sugar as well as on bodyfat to be responsible for the correlation found. In order to check whether this was the case the effect of age was studied on the cor relation between bodyfat and blood sugar in different age classes. Table 11 shows a positive correlation be- tween bodyfat and blood sugar level after ingestion of glucose in men aged 40—59 and 60—79 and in women aged 40—59 In younger men and older women no such tendency was found, while among younger women the correlation appeared to be neg ative.

In an attempt to explain the situa tion in younger persons reference may be made to a differentiation into ac tive and static obesity proposed by BEAUDOIN et al. (1953) According to these authors, increasing, active obes- ity is accompanied by a tendency to- wards lower glucose tolerance test curves.

In the men, but not in the women, increased fatfree bodyweight as well as increase of stature and skeletal sturdiness expressed as condylar breadth, respectively biatyloid breadth was accompanied by a lowering of the sugar level which was largest at 90 minutes after ingestion of glucose This may be compared with the de cline of the curve during the 30—90 minute period which was found to be positively correlated with fatfree weight and then with as well stature as sturdiness (Table 12).

The fall of the glucose level during the 30—90 minute period can be com pared with a corresponding fall in intravenous glucose tolerance test. In series C it had been shown (NILSSON 1962) that the rate of the fall, ex

Table 10 *Correlation between various anthropometric factors and blood sugar level fasting and at various intervals after glucose administration.*

Blood sugar		Weight	Height	Condyl. breadth	F free weight	Bodyfat	Age
0	Total	.29	— 01	14	09	.20	.23
	M	.35	— 06	03	— .02	40	.27
	F	.23	— 15	17	05	14	.27
30	Total	25	.12	.20	17	.07	.31
	M	17	— 06	.01	— 05	.22	.39
	F	.23	.01	16	12	13	.33
60	Total	25	— 02	11	.05	15	.50
	M	17	— .26	— .09	— .22	.39	.53
	F	.30	— .01	19	13	17	45
90	Total	18	— 17	— 01	— 11	.27	47
	M	10	— .30	— 13	— .25	31	48
	F	.34	— 04	.21	16	.31	45
120	Total	13	— 15	— 04	— 12	23	.36
	M	16	— 19	— 05	— 16	.30	.34
	F	.20	— 08	15	10	11	.29
150	Total	15	— 10	04	— 00	11	17
	M	.22	— 04	01	— 00	.25	11
	F	12	— 20	10	.05	05	.23
180	Total	10	— 04	05	0.4	05	00
	M	20	10	07	09	12	— 07
	F	03	— .20	0	.00	— 03	09

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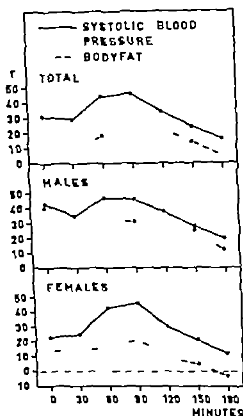


Fig. 2. Variation in shape of glucose curve with systolic blood pressure respectively body fat at different intervals during glucose tolerance test.

diet the risk of development of diabetes mellitus.

To test its value for predicting this risk various methods may be used

1. A longitudinal investigation with registration of the development of confirmed diabetes compared with the results of the tolerance test.
2. Demonstration of bimodality of the distribution curve. A second peak with elevated blood sugar values might be thought to correspond to persons with heredity for the disease.
3. Calculation of the number of prospective diabetics in different groups with a known risk of developing diabetes and comparison of the distributions of the glucose tolerance values in these groups.

As yet the present material can only be utilized for analysis according to point 3.

Of this sample of 300 persons, 2 men and 6 women had already developed diabetes. The frequency of diabetes and the degree of manifestation in different age classes is known for the population from which the samples was derived (SILWER 1958, NILSSON 1962). Diabetes must be expected in 2.1 % of the males and 4.8 % of the females below 80 years of age. Table 14 gives the number of expected and observed diabetics

Table 13. Number of persons with concordance of blood sugar 120 minutes after administration of glucose (B.S.), bodyfat (B.F.), and systolic blood pressure (S.B.P.). Concordance was said to be present when the values compared deviated in the same direction from the age-corrected mean value.

	Concordance	Non-concordance
B.S. — B.F	119	86
B.S. — S.B.P	118	87
B.F — S.B.P	114	91
B.S. — B.F — S.B.P	72	
B.S. — B.F but not S.B.P	45	
B.S. — S.B.P but not B.F	41	
S.B.P — B.F but not B.S.	41	

Table 11 *Coefficients of correlation between anthropometric factors and blood sugar after 60 and 120 minutes in males and females grouped according to age*

	Weight		Fatfree weight		Body fat	
	Bls 60'	Bls 120'	Bls 60'	Bls 120'	Bls 60'	Bls 120'
Total	.25	13	05	— 12	18	.23
Males	17	17	—.21	— 16	.32	.30
Females	.30	.20	13	10	17	11
M 20—39	— 10	—.08	— 15	— 04	— 02	— 10
40—59	26	15	— 15	— 14	.35	.30
60—79	01	18	—.27	— 18	.31	.32
F 20—39	— 10	—.07	12	.28	—.32	—.26
40—59	.38	.24	— 20	—.20	42	.33
60—79	31	.23	.36	18	— 04	00

pressed as K value, is correlated with the amount of fatfre weight

EFFECT OF FACTORS VARYING WITH THE BLOOD PRESSURE

The glucose level was positively cor related with the blood pressure throughout the tolerance test (See Fig 2) The strength of the correlation as well as of that between the blood sugar and the bodyfat was strongest at 60—120 minutes Since bodyfat and blood pressure are intercorrelated (Table 22) it is difficult to distinguish between the influence, on the blood

Table 12 *Coefficients of correlation between anthropometric factors and fall in blood sugar level in the interval between 30 min. and 90 min. respectively between 30 min. and 120 min. after administration of glucose*

	diff 30'—90'	diff 30'—120'
Weight	04	11
Height	.22	.20
Condylar breadth	.22	18
Fatfree weight	.23	.21
Bodyfat	— 18	— 09

sugar of factors varying with bodyfat and factors varying with the blood pressure.

In an attempt to solve this problem the covariation of the 120 minute blood sugar level bodyfat and systolic pressure were studied. The results are given in Table 13 which shows that when bodyfat and systolic blood pres sure deviated in the same direction the corresponding value of the blood sugar also deviated in the same di rection from its mean value in 72 cases and in the opposite direction in 44 cases while when bodyfat and blood pressure deviated in opposite directions the influence of these fac tors on the blood sugar level were of the same magnitude.

GLUCOSE TOLERANCE AS MEA SURE OF THE RISK OF DEVE LOPMENT OF DIABETES MELLITUS

The glucose tolerance test has been used especially in an attempt to pre

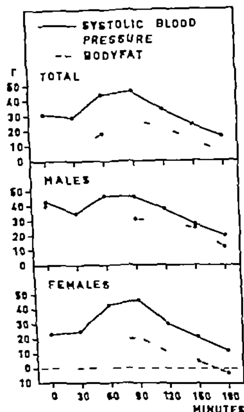


Fig. 2. Variation in shape of glucose curve with systolic blood pressure respectively body fat at different intervals during glucose tolerance test.

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To test its value for predicting this risk, various methods may be used:

1. A longitudinal investigation with registration of the development of confirmed diabetes compared with the results of the tolerance test.
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	Concordance	Non-concordance
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B.F. — S.B.P.	114	91
B.S. — B.F. — S.B.P.	72	
B.S. — B.F. but not S.B.P.	45	
B.S. — S.B.P. but not B.F.	44	
S.B.P. — B.F. but not B.S.	44	

Table 14 Expected and observed numbers of diabetics in different age classes

Age	Males		Females		Total	
	exp.	found	exp.	found	exp.	found
20—39	0.2	0	0.2	0	0.4	0
40—59	0.4	0	0.4	1	0.8	1
60—79	0.8	2	1.2	5	2.0	7
Total	1.4	2	1.6	6	3.2	8

classified according to sex and age. *An overmorbidity is evident among* old females with slight diabetes treated by tablets, but since the sample series was small the figures may be due to chance.

One may expect about 8 out of every 300 persons to develop clinically manifest diabetes in the course of their life. It therefore seems unreasonable to expect more than 3 or 4 of the 207 (non diabetic) persons examined with the glucose tolerance test to develop diabetes later in life.

Among those who will later develop the disease, one may expect above all close relatives of diabetics—compare previous analysis of series C (NILSSON 1962).

Of the 207 persons examined 26 i.e. 12.5 % were close relatives of diabetics (children, grand-children, nieces, nephews, siblings, parents). This percentage was the same as that (12.6 %) found by JORDE (1962) in another *Scandinavian series of 4 682* persons though his material included also persons with diabetic cousins. The corresponding figure for series C—18 year old men—was 8.9 %.

The distribution of the fasting blood sugar levels among these persons compared with that of the sample as a whole is given in Fig 3. Table 15 compares the blood sugar values found at different intervals of the *tolerance test in relatives of diabetics* with corresponding means for age in the entire sample.

Table 15 shows that in relatives of *diabetics the blood sugar level tended* to be high both before and during the tolerance test but only in the age groups *40—59* and *60—79* years.

Since the influence of bodyfat on the glucose tolerance test is marked it was checked whether increased bodyfat in relatives with high fasting values and high values at 120 minutes might explain the values found. Subsequent analysis revealed no evidence

Table 15 Distribution of blood sugar levels fasting and at different intervals of the test in close relatives of diabetics compared with mean for corresponding age group in entire sample (=denotes mean fasting blood sugar level ± 1.5 mg/100 ml and for the other mean blood sugar levels ± 5 mg/100 ml)

Age	Blood sugar level	0	30	60	90'	120'	150'	180'
20—39	+	4	4	2	3	2	2	2
	=	1	1	4	2	4	2	4
	—	3	3	3	3	3	4	2
40—59	+	9	7	9	6	6	5	3
	=	1	3	0	4	2	3	6
	—	1	1	2	1	3	3	2
60—79	+	4	5	4	4	4	3	4
	=	0	1	1	1	0	2	0
	—	3	1	2	2	2	2	3
Total	+	17	16	15	13	12	10	9
	=	2	5	5	7	6	7	10
	—	7	5	6	6	8	9	7

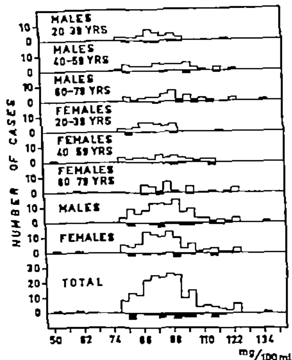


Fig 3. Distribution of fasting blood sugar levels in close relatives (filled columns) of diabetics compared with that of entire sample.

in support of such an assumption (Table 16)

In a corresponding way the distribution of the glucose values was studied among other categories with an expected increased risk of diabetes namely among 27 persons who had had gallstone disease, 20 persons with parents who had had cerebral hemorrhage 13 women who had given birth to children weighing more than 4 000 g, 11 women who had borne more than 3 children. Here no tendency to higher values was noted. (Table 17)

Table 16. *Distributio f fasting blood sugar and blood sugar 120 minutes after ingestion of glucose in relative f diabetics grouped according t bodyfat above below the age-corrected mean value (=denotes age-corrected mean ± 1.5 % f bodyweight re pect vely for fasting blood sugar mean ± 1.5 mg/100 ml and f blood sugar 120′ mean ± 5 mg/100 ml).*

Body fat relation of diabetics		Fasting blood sugar			Blood sugar 120′		
		+	=	−	+	=	−
+	17	11	1	5	9	3	5
=	5	3	1	1	2	1	2
−	4	3	0	1	1	3	1

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RATE OF FALL OF GLUCOSE CURVE

So-called oxyhyperglycemic blood sugar curve, *i.e.* early maximum value followed by rapid and marked fall, is said to be common in patients with peptic ulcer and particularly after gastric resection.

Table 14 *Expected and observed numbers of diabetics in different age classes*

Age	Males		Females		Total	
	exp.	found	exp.	found	exp.	found
20—39	0.2	0	0.2	0	0.4	0
40—59	0.4	0	0.4	1	0.8	1
60—79	0.8	2	1.2	5	2.0	7
Total	1.4	2	1.8	6	3.2	8

classified according to sex and age. An overmorbidity is evident among old females with slight diabetes treated by tablets but since the sample series was small the figures may be due to chance

One may expect about 8 out of every 300 persons to develop clinically manifest diabetes in the course of their life It therefore seems unreasonable to expect more than 3 or 4 of the 207 (non diabetic) persons examined with the glucose tolerance test to develop diabetes later in life

Among those who will later develop the disease, one may expect above all close relatives of diabetics—compare previous analysis of series C (NILSSON 1962)

Of the 207 persons examined 26 *i e* 12 5 % *were close relatives of* diabetics (children, grand-children nieces, nephews, siblings, parents) *This percentage was the same as that* (12 6 %) found by JORDE (1962) in another Scandinavian series of 4 682 *persons though his material included* also persons with diabetic cousins. The corresponding figure for series C—18 year old men—was 8 9 %

The distribution of the fasting blood sugar levels among these persons compared with that of the sample as a whole is given in Fig 3 Table 15 compares the blood sugar values found at different intervals of the tolerance test in relatives of diabetics with corresponding means for age in the entire sample

Table 15 shows that in *relatives of diabetics the blood sugar level tended to be high both before and during the* tolerance test, but only in the age groups 40—59 and 60—79 years.

Since the influence of bodyfat on the glucose tolerance test is marked it was checked whether increased bodyfat in relatives with high fasting values and high values at 120 minutes might explain the values found Subsequent analysis revealed no evidence

Table 15 *Distribution of blood sugar levels fasting and at different intervals of the test in close relatives of diabetics compared with mean for corresponding age group in entire sample (= denotes mean fasting blood sugar level ± 1.5 mg/100 ml and for the other mean blood sugar levels ± 5 mg/100 ml)*

Age	Blood sugar level	0	30'	60'	90'	120'	150'	180'
	+	4	4	2	3	3	2	3
20—39	=	1	1	4	2	4	2	4
	−	3	3	2	3	2	4	3
	+	9	7	9	6	6	5	3
40—59	=	1	2	0	4	2	3	6
	−	1	1	2	1	3	3	3
	+	4	5	4	4	4	3	4
60—79	=	0	1	1	1	0	2	0
	−	3	1	2	2	3	2	3
	+	17	18	15	13	12	10	9
Total	=	2	3	5	7	6	7	10
	−	7	5	6	6	8	9	7

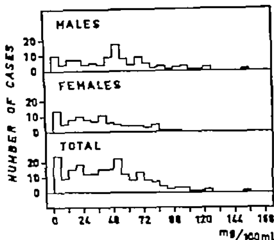


Fig. 4. Distribution of fall in blood sugar level in the interval between 30 and 90 minutes after ingestion of glucose.

Table 18. *Distribution according to degree of glycosuria and age.*

Glycosuria g/100 ml	Age	20—39		40—59		60—79		Total	
Males		31		42		36		109	
0		24	77 %	21	50 %	23	64 %	68	63 %
0.1—0.2		4	13 %	10	24 %	4	11 %	18	16 %
0.3—0.5		1	3 %	10	24 %	6	17 %	17	16 %
> 0.5		2	6 %	1	2 %	3	8 %	6	5 %
Females	n	34		36		28		98	
0		30	88 %	33	92 %	20	71 %	83	85 %
0.1—0.2		2	6 %	2	6 %	6	21 %	10	10 %
0.3—0.5		2	6 %	1	3 %	2	7 %	5	5 %
> 0.5								0	0

Table 19. *Distribution of fasting blood sugar levels among persons grouped according to degree of glycosuria.*

	Glycosuria / Fasting bloodsugar	0	0.1—0.2 %	0.3—0.5 %	> 0.5 %
Males	< mean + 1 SD	63	18	17	6
	≥ mean + 1 SD	4	1	4	0
	≥ mean + 2 SD	1	—	1	2
Females	< mean + 1 SD	73	10	5	—
	≥ mean + 1 SD	8	3	3	—
	≥ mean + 2 SD	2	—	—	—

Table 17 *Distribution of blood sugar levels among*

1. persons who had suffered from gallstone.
2. persons whose father or mother had cerebral haemorrhage.
3. women who had borne children weighing more than 4 000 g.
4. women who had borne more than 3 children.

(= denotes mean value ±1.5 mg/100 ml fasting blood sugar respectively ±5 mg/ml for other blood sugar values.)

		Cholelith.	Hered. cerebral hemorrh.	> 4,000 g	> 3 children
0	+	15	6	8	4
	=	2	2	1	0
	−	10	10	4	7
30 min	+	10	7	3	2
	=	7	6	4	4
	−	10	7	6	5
60 min	+	12	8	3	3
	=	4	2	3	2
	−	11	10	7	6
90 min	+	13	3	2	4
	=	7	2	5	2
	−	7	10	6	5
120 min.	+	12	10	5	3
	=	5	2	5	1
	−	10	6	3	7

Of the persons examined 11 had had peptic ulcer including one who had undergone gastric resection. This patient a 56 year old man had a typical oxyhyperglycemic curve with a blood sugar of 175 mg/100 ml at 60 minutes and of only 55 mg/100 ml at 90 minutes. Of the remaining patients who had had peptic ulcer the distribution of the curves was normal.

It is clear from the distribution of the fall of the curves during the 30—90 minute period (Fig 4) that here we are dealing with a continuous distribution.

GLYCOSURIA FOLLOWING INGESTION OF GLUCOSE

The excretion of sugar in the urine following ingestion of glucose differed markedly with sex. The frequency of glycosuria was higher and the amount of glucose excreted was clearly larger in the men than in the women. The frequency of glycosuria was not found to vary significantly with age (see Table 18)

The distribution of the fasting blood sugar level among persons with varying degree of glycosuria is given (Table 19) to enable evaluation of the relationship between glycosuria and fasting blood sugar The fasting blood sugar levels are grouped according to the age-corrected mean value +1 S D and +2 S D It is seen that large amounts of urinary sugar are correlated with higher fasting blood sugar levels, but this rule was not without exceptions, for high fasting blood sugar levels—in one case also outside the mean +2 S.D —were also seen in the absence of glycosuria

SUMMARY

Analysis of the results of the oral glucose tolerance test appeared to show as follows

1 The fasting blood sugar level as well as the level of the blood sugar curve varies with age particularly regarding the values noted at 60 and 90 minutes.

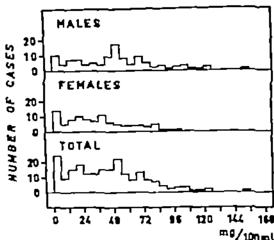


Fig. 4 Distribution of fall in blood sugar level in the interval between 30 and 90 minutes after ingestion of glucose.

Table 18. *Distribution according to degree of glycosuria and age.*

Glycosuria g/100 ml	Age	[illegible]		[illegible]		[illegible]		Total	
Males		31		42		36		109	
0		24	77 %	21	50 %	23	64 %	68	63 %
0.1—0.2		4	13 %	10	24 %	4	11 %	18	16 %
0.3—0.5		1	3 %	10	24 %	6	17 %	17	16 %
> 0.5		2	6 %	1	2 %	3	8 %	6	5 %
Females	n	34		36		28		98	
0		30	88 %	34	92 %	20	71 %	84	85 %
0.1—0.2		2	6 %	2	6 %	6	21 %	10	10 %
0.3—0.5		2	6 %	1	3 %	2	7 %	5	5 %
> 0.5								0	0

Table 19. *Distribution of fasting blood sugar levels among persons grouped according to degree of glycosuria.*

	Glycosuria / Fasting bloodsugar	0	0.1—0.2 %	0.3—0.5 %	> 0.5 %
Males	< mean + 1 SD	68	18	17	6
	≥ mean + 1 SD	4	1	4	0
	≥ mean + 2 SD	1	—	1	2
Females	< mean + 1 SD	83	10	5	—
	≥ mean + 1 SD	8	3	3	—
	≥ mean + 2 SD	2	—	—	—

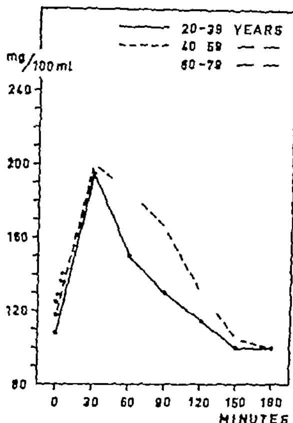


Fig 5. Upper limits of normal distribution in various age groups of glucose values after administration of 30 g glucose/m body surface found on examination of capillary blood.

2 In the higher age classes a positive correlation exists between the relative amount of bodyfat and the level of the blood sugar curve, the correlation being strongest at 60 and 90 minutes

3 *In males the height of the glucose* curve is negatively correlated with fatfree weight, stature and condylar breadth. The correlation is most marked at 90 minutes.

4 Factors varying with the blood pressure are positively correlated with the *blood sugar level even* after elimination of the effects of age and body fat

5 At ages above 40 years the fasting blood sugar level and the level after ingestion *of glucose tend to* be elevated in close relatives of diabetics

6 Glycosuria *following ingestion of glucose is much more common in* males than in females.

CONCLUSION

*In the evaluation of oral glucose toler*ance curves to demonstrate diabetes mellitus or the risk of developing *this disease it appears necessary to take age into account. Therefore a* curve based on the mean value +2 SD at different intervals of the test was constructed for different age groups (Fig 5 Table 20) Not until several values fall outside the curve *in such a way as cannot be ascribed to disturbed absorption during the* test does there appear to be reason *to assume* diabetes mellitus—but even then only after taking into account *bodybuild and other relevant factors* such as *state of* nutrition, physical *fitness* physical exertion before the examination, *the time of day* etc.

Table 20 *Upper limit (mean+2 S.D) of glucose level (mg/100 ml) fasting and at various intervals after administration of glucose in persons grouped according to age*

Time \ Age	20–39 years	40–59 years	60–79 years
0	108	118	125
30	195	200	215
60	160	190	210
90	130	165	210
120′	115	130	170
150′	100	103	125
180	100	100	110

Blood pressure

The variation of the blood pressure with age (Table 21) coincided with what has been demonstrated previously for males and females. As known (FABER 1924 BØE et al. 1957) there is a correlation between the blood pressure and bodyweight. This is probably due mainly to the correlation between blood pressure and bodyfat, which has been shown by evaluation of bodyfat by the skinfold technique (LINDEGÅRD 1957 TRUEDSSON 1962) and direct studies on the subcutaneous fat (BJURULF 1959)

Table 22 shows the correlation of the anthropometric values with the blood pressure.

The correlation between the blood pressure and bodyfat is marked in all age groups with the exception of old women, where the blood pressure continues to increase after 70 years of age even when the amount of bodyfat has began to diminish.

In order to distinguish between the effect of age and that of bodyfat on blood pressure, bodyfat was compared with the systolic blood pressure after the values noted for both factors had been corrected for age.

Table 23 shows that even after such correction for age a positive correlation was found between bodyfat and blood pressure in females but not with certainty in males.

The blood pressure recorded by the auscultatory method probably does not deviate from that obtained by direct measurement, not even in obese person (RIES 1960)

Table 21 *Blood pressure in males and females / Series 4*

		20—39 years			40—59 years			60—79 years	
		mean	SD		mean	SD		mean	SD
Males									
S. B. P (lying)	31	127.7	13.4	42	142.3	14.9	36	161.4	22.5
S. B. P (after standing 3 min)	31	124.0	14.0	42	137.4	16.5	36	157.9	24.3
D. B. P (lying)	31	76.6	7.8	42	84.1	8.8	36	90.3	11.8
Females									
S. B. P (lying)	34	130.5	14.8	37	142.8	17.9	28	173.4	21.0
S. B. P (after standing 3 min)	34	123.3	15.4	37	140.3	17.9	28	167.3	21.1
D. B. P (lying)	34	79.9	8.3	37	83.0	7.6	28	93.3	9.5

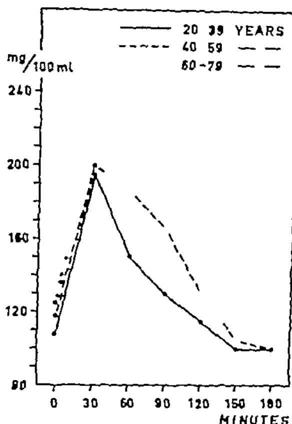


Fig 5 Upper limits of normal distribution in various age groups of glucose values after administration of 30 g glucose/m body surface found on examination of capillary blood.

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3 In males the height of the glucose curve is negatively correlated with fatfree weight, stature and condylar breadth. The correlation is most marked at 90 minutes.

4 Factors varying with the blood pressure are positively correlated with the blood sugar level even after elimination of the effects of age and body fat

5 At ages above 40 years the fasting blood sugar level and the level after ingestion of glucose tend to be elevated in close relatives of diabetics.

6 Glycosuria following ingestion of glucose is much more common in males than in females.

CONCLUSION

In the evaluation of oral glucose tolerance curves to demonstrate diabetes *mellitus or the risk of developing* this disease it appears necessary to take age into account. Therefore a curve based on the mean value +2 S D at different intervals of the test was constructed for different age groups (Fig 5 Table 20) Not until several values fall outside the curve in such a way as cannot be ascribed to disturbed absorption during the test does there appear to be reason to assume diabetes mellitus—but even then only after taking into account bodybuild and other relevant factors such as state of nutrition physical fitness physical exertion before the examination, the time of day etc.

Table 20 *Upper limit (mean+2 S.D) of glucose level (mg/100 ml) fasting and at various intervals after administration of glucose in persons grouped according to age*

Time \ Age	20–39 years	40–59 years	60–79 years
0	108	118	135
30′	195	200	215
60′	150	190	210
90′	130	165	210
120	115	130	170
150	100	105	135
180	100	100	110

Correlation found between pulse-frequency duration of PQ and QT as well as the height of R_V are given in Table 24 from which it is clear that the QT varies more closely than PQ with pulse frequency

Table 25 shows that the pulse frequency varies with sex and age (cf AVERILL & LAMB 1960) The low pulse frequency is the main explanation for the somewhat longer QT waves noted in the men. QT also seems to be prolonged by an age-correlated factor—despite increasing pulse frequency the duration of the QT wave appeared to increase with increasing age.

The difference of the PQ with sex cannot be explained by difference in pulse frequency

On analysis of the correlation between pulse frequency and bodybuild (Table 26) a tendency to a positive correlation was found between the pulse frequency and bodyfat and a

Table 24 *Coefficients of correlation between pulse rate duration of PQ and QT and height of* R_{V4}

	PQ	QT	R_{V4}
Pulse rate			
Total	−.35	−.46	.04
Males	−.24	−.60	−.02
Females	−.31	−.57	.20
PQ		.27	.04
		.15	−.14
		.30	.06
QT			−.02
			.02
			−.10

Table 25. *Pulse rate (beats/min) duration of PQ and QT (sec.) and height of* R_{V4} *(mm) in Series A.*

			20—39 years			40—59 years			60—79 years	
			mean	SD		mean	SD		mean	SD
Pulse rate	Males	31	60.7	8.4	42	61.6	9.6	36	64.6	12.0
	Females	34	64.9	12.8	37	69.7	12.1	28	71.7	12.0
PQ	Males	31	0.160	0.020	42	0.158	0.021	36	0.160	0.023
	Females	34	0.137	0.024	37	0.139	0.026	28	0.144	0.025
QT	Males	31	0.388	0.028	42	0.387	0.030	36	0.391	0.034
	Females	34	0.375	0.030	37	0.379	0.035	28	0.378	0.032
R_V	Males	31	10.1	5.6	42	10.8	7.5	36	15.2	8.4
	Females	34	12.6	3.6	37	13.0	6.0	28	14.3	3.8

Table 22 *Correlation between systolic (S) and diastolic (D) blood pressure and various anthropometric measurements*

	Weight		Height		Condylar breadth		Fatfree weight		Body fat		Body surface	
	S	D	S	D	S	D	S	D	S	D	S	D
Total	.21	.23	—.16	—.08	12	10	—.03	01	.26	.24	.10	14
Males	.22	.34	—.26	—.06	09	17	—.13	05	.32	.33	.08	.25
Females	.24	.16	—.13	—.10	.22	13	06	04	.23	17	18	.31
M 20—39	.27	44	—.04	.11	17	42	10	.37	.21	.22	.19	.39
40—59	07	.17	—.12	02	—.05	—.03	—.24	—.11	.25	.26	05	.32
60—79	17	.31	—.12	13	11	.21	—.08	16	.25	.25	.05	.36
F 20—39	40	.27	13	.09	.19	.23	.27	.22	.25	.31	.38	.27
40—59	14	16	—.25	—.13	01	11	—.30	—.13	.45	.25	.08	.13
60—79	—.06	—.37	—.10	—.15	18	—.19	01	—.20	—.15	—.28	—.09	—.28

Table 23 *Distribution of persons according to bodyfat and systolic blood pressure corrected for age (+ above and – below mean for age).*

	Body fat	Blood press.	Age 20—29	30—39	40—49	50—59	60—69	70—79	Total
Males	+	+	2	6	5	5	9	3	30
	+	—	5	4	4	8	3	4	28
	—	+	3	3	6	5	5	4	25
	—	—	3	5	6	5	7	1	26
Females	+	+	4	7	5	6	6	3	31
	+	—	3	4	3	3	6	1	20
	—	+	3	4	3	3	4	1	18
	—	—	3	6	6	8	4	3	30
Total	+	+	6	13	10	11	15	6	61
	+	—	8	8	7	11	9	5	48
	—	+	6	7	8	8	9	5	43
	—	—	6	11	11	13	11	4	56

Body temperature is, on the average, somewhat higher in females than in males (See Table 28.)

A correlation between anthropometrical measurements and temperature could only be demonstrated regarding bodyfat, especially in young and middle-aged men, (Table 29) This correlation appeared to decrease with increasing age in both sexes.

Tabl 28. *Body t mperature in Series A.*

		20—39 years mean	SD		40—59 years mean	SD		60—79 years mean	SD
Males	31	36.92	0 23	42	36.73	0.24	36	36.82	0.29
F males	34	37 16	0.25	37	36.94	0.30	28	36.89	0.29

Table 29 *Correlation between body temperature and body fat in males respec tively f male grouped according t age.*

Total	.29
Males	.35
Females	.09
M 20—39	.41
40—59	.30
60—79	.23
F 20—39	.26
40—59	.18
60—79	—.03

Table 26 *Coefficients of correlation between pulse rate duration of PQ and QT height of R_{V4} and some anthropometric measurements systolic blood pressure and body temperature*

		Fatfree weight	Height	Condyle breadth	Body fat	S B P	Temperature
Pulse rate	*Total*	—.30	—.23	—16	19	.31	12
	Males	—12	—02	10	.20	.21	.29
	Females	—13	02	05	09	.22	.25
	M 20—39	01	—11	12	.28	—03	.2
	40—59	—.23	—05	13	14	.20	.50
	60—79	—09	—04	02	18	14	13
	F 20—39	.33	.30	14	.26	.32	.33
	40—59	—12	—07	—.08	18	.32	47
	60—79	—11	—.30	10	—08	—21	45
PQ	Males	01	01	02	00	—15	.03
	Females	11	.20	15	—05	06	—.30
QT	Males	18	07	11	—18	—06	—.23
	Fem les	17	13	15	—01	00	—.27
R_{V4}	Males	00	06	.03	00	.00	—05
	Females	06	07	12	—08	18	93

negative correlation between pulse *frequenc and fatfree weight Exceptions to this rule were the group of* elderly women in whom bodyfat might have begun to decrease and the pulse frequency had continued to in crease In younger women a positive correlation was found between the pulse frequency and fatfree weight which appeared to be due mainly to a positive correlation between pulse *frequency and stature.*

No substantial correlation was found between PQ QT and Rv and bodyweight *except what could be* ascribed to the influence of body weight on pulse frequency

The *distribution of abnormalities* found in the electrocardiograms ac cording to BLACKBURN et al (1960) is given in *Table 27*

Table 2 *Electrocardiographic changes in males and females classified according to* BLACKBURN *et al (1960).*

	Males	Females	Total
Left axis deviation ($S_{II} > R_{II}$)	8	1	9
$R_{V5, V6} > 26$ mm	6	0	6
ST-depression < 0.5 mm with reciprocal depression f ST	0	2	2
AV block I(PR > 0,21)	2	1	3
Incomplet right bundle branch block	1	0	1
Frequent (4 or more per *10 compelxes) supra entr* or ventr extra syst les	2	0	2
Auricular fibrillation	0	1	1
Frequency > 100 min	1	3	4
Frequency < 50 min	6	1	7
Low voltage (no positive or negative wave over 5 mm in lead I—III)	3	0	3
T > 12 mm in one of more of leads I—III	5	0	5

Body temperature

Body temperature is, on the average, somewhat higher in females than in males. (See Table 28.)

A correlation between anthropomet rical measurements and temperature could only be demonstrated regarding bodyfat, especially in young and middle-aged men, (Table 29) This correlation appeared to decrease with increasing age in both sexes.

Table 28. *Body t mperatur in Series A.*

		20—39 years			40—59 years			60—79 years	
		mean	SD		mean	SD		mean	SD
Males	31	36.92	0.33	42	36.75	0.34	36	36.82	0.29
Females	34	37.18	0.35	37	36.96	0.30	28	36.89	0.29

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Total	.29
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Females	.09
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60—79	.25
F 20—39	.36
40—59	.18
60—79	—.03

Table 26 Coefficients of correlation between pulse rate duration of PQ and QT height of R_{V4} and some anthropometric measurements systolic blood pressure and body temperature

		F tfree weight	Height	Condylar breadth	Body fat	S. B. P	Temperature
Pulse rate	Total	— .30	— .23	— .16	.19	.31	42
	Males	— 13	— 02	10	.20	.31	.39
	Females	— 13	02	06	09	.32	.33
	M 20—39	01	— 11	12	28	— 03	.27
	40—60	— .23	— 05	13	14	.29	.30
	60—70	— .09	— 04	02	18	14	43
	F 20—39	.23	.30	14	26	.32	.33
	40—59	— 12	— 07	— 08	18	.32	47
	60—79	— 11	— 30	10	— 03	— 21	43
PQ	Males	01	01	02	00	— 15	.03
	Females	.24	.20	16	— .05	06	— .30
QT	Males	18	07	11	— 18	— 06	— .33
	Females	17	13	15	— 01	00	— .37
R_{V4}	Males	00	.06	03	00	00	— .05
	Females	06	07	13	— .08	16	05

negative correlation between pulse frequency and fatfree weight Exceptions to this rule were the group of elderly women in whom bodyfat might have begun to decrease and the pulse frequency had continued to increase. In younger women a positive correlation was found between the pulse frequency and fatfree weight which appeared to be due mainly to a positive correlation between pulse frequency and stature.

No substantial correlation was found between PQ QT and R_V and bodyweight except what could be ascribed to the influence of body weight on pulse frequency

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ST-depression < 0.5 mm with arcuat depression of ST	0	2	2
AV block I(PR > 0,21)	2	1	3
Incomplet right bundl branch block	1	0	1
Frequ nt (4 or more per 40 complexes) supraventr or ventr extrasystoles	2	0	2
Auricular fibrillation	0	1	1
Frequency > 100/min	1	3	4
Frequency < 50/min	8	1	9
Low voltag (no positive or negative wave ove 5 mm in lead I—III)	3	0	3
T > 12 mm 1 one or more *of leads I—III*	5	0	5

Table 22. *Correlation between body-build and different types of blood cells.*

		Hemoglobin	Erythrocytes	Leucocytes	Poly-nuclear	Mono-nuclear
Weight	M	.21	.04	— .05	— .10	.03
	F	.06	— .01	.04	.07	.03
Height	M	.08	.03	— .18	— .22	— .03
	F	.00	.02	.06	.04	.06
Condylar breadth	M	.13	.00	— .16	— 14	— .18
	F	.01	— .01	.03	.09	.01
Fat-free weight	M	.07	.05	— .25	— .28	— .11
	F	.01	— .05	07	.10	— .06
Bodyfat	M	.19	.06	.06	.05	.11
	F	.07	— .03	.00	— .02	.03

The numbers of erythrocytes and poly nuclear and mononuclear leucocytes tended to vary with one another (Table 30)

The number of erythrocytes and the amount of hemoglobin were substantially smaller in females (Table 31)

The hemoglobin and the number of erythrocytes in males tended to decrease with age while in females the opposite tendency was noted.

In both men and women the number of leucocytes increased with age, which was due mainly to an increased number of polynuclear cells.

No definite correlation was found between hematological data and body build (Table 32)

Table 30 *Correlation between frequencies of different types of blood cells.*

	Leucocytes	Poly nuclear	Mono-nuclear
Erythrocytes	17	11	.19
Leucocytes		.92	.69
Polynuclear			.36

Table 31 *Hemoglobin and number of different types of blood cells in Series A.*

	20—39 years			40—59 years			60—79 years		
	n	mean	SD		mean	SD		mean	SD
Males									
Hb (g/100 ml)	31	15.47	0.84	42	15.27	0.93	36	14.79	1.20
Erythroc. (mill.)	31	4.68	0.26	42	4.67	0.33	36	4.54	0.30
Leucocytes	31	5940	2020	42	6670	2120	36	6810	1660
Polynuclear	31	3660	1440	42	4170	1740	36	4610	1410
Mononuclear	31	2280	890	42	2500	690	36	2170	900
Females									
Hb (g/100 ml)	34	13.39	0.97	37	13.18	1.09	28	13.68	1.02
Erythroc. (mill.)	34	4.22	0.27	37	4.15	0.32	28	4.33	0.33
Leucocytes	34	5120	1240	37	5770	1600	28	5990	2160
Polynuclear	34	3140	990	37	3510	1260	28	3780	1650
Mononuclear	34	1980	570	37	2260	830	28	2210	560

Table 22. *Correlation between body-build and different types of blood cells.*

		Hemoglobin	Erythrocytes	Leucocytes	Polynuclear	Mononuclear
Weight	M	.31	.06	— .05	— .10	.03
	F	.06	— .01	.06	.07	.03
Height	M	.06	.03	— .18	— .22	— .03
	F	.00	.02	.06	.04	.06
Condylar breadth	M	13	.00	— .16	— .14	— .18
	F	.01	— .01	.08	.09	.01
Fat-free weight	M	.07	.05	— .25	— .28	— .11
	F	.01	— .06	.07	.10	— .06
Bodyfat	M	.19	.06	.08	.05	.11
	F	.07	— .03	.00	— .02	.03

As is apparent from Table 34 showing data about series A and C, a negative correlation was found between albumin and various globulins. A strong positive correlation is seen between α_1 and α_n, respectively β_1 and β_n, and is also obvious in the total group α_n, β β and γ globulins.

The E.S.R. was negatively correlated with albumin and positively correlated with various globulin fractions. These correlations could be compared with the demonstrated E.S.R. increasing "agglomerin property of haptoglobin, coeruloplasmin (RUHENSTROTH BAUER et al. 1962) and 7 S-gammaglobulin (BRITTINGER 1963).

A striking negative correlation was found between the amount of albumin and age (Table 35). Of the globulin fractions α_2 and β_1 were most strongly positively correlated with age.

Table 35 also shows a correlation between the E.S.R. and bodybuild. A

Table 35 *Erythrocyte sedimentation rate (mm) and electroforetic fractions of blood proteins (mg/100 ml) in Series A.*

	20—39 years			40—59 years			60—79 years		
	n	mean	SD	n	mean	SD		mean	SD
Males ESR	31	3.7	2.6	42	7.6	6.2	36	16.8	12.8
Alb.		3 75	0.34		3.78	0.39		3.56	0 38
α		0 31	0.05		0.32	0.07		0.30	0 03
α_2		0 47	0.10		0.56	0.14		0.68	0.13
β		0.47	0 07		0.55	0 13		0.55	0 15
β_1		0.28	0 10		0.34	0 08		0.41	0 18
γ		1 12	0 15		1 12	0.28		1 23	0 19
Females ESR	34	8.8	6.2	37	16.7	10.6	28	23.3	21 6
Alb.		3 89	0 28		3.69	0.22		3.81	0.31
α_1		0.32	0.06		0.35	0.06		0.34	0.09
α_2		0.50	0.08		0.55	0 14		0.53	0 13
β_1		0 48	0.11		0.52	0 12		0.48	0 08
β		0.24	0 07		0.33	0 15		0.32	0.13
γ		1 12	0.23		1.27	0.20		1 15	0.29

Table 34. *Correlations between E.S.R and various electrophoretic fractions, and between various electrophoretic fracti ns.*

	E.S.R.	Alb.	α_1	α_2	β_1	β_2
Alb.						
Total	—.19					
Males	—.04					
Females	—.23					
α_1	.26	—.18				
	.03	.00				
	.29	—.35				
		—.29				
α_2	.26	.00	.32			
	.31	19	.23			
	.27	—.19	41			
		—.35*	44			
β_1	18	—14	19	48		
	.18	—01	.29	49		
	.23	—.28	19	44		
		—16*	.25	.27*		
β_2	17	—.26	13	43	.59	
	.35	—12	.10	31	.60	
	17	—.27	23	.53	87	
		—30*	.29*	.33*	.30*	
γ	29	—03	.08	.30	.29	.28
	.24	01	.04	32	.28	37
	.30	—11	07	.11	18	.23
		—.28*	.08	30*	.20*	.27*

Series C.

positive correlation with bodyfat was observed at least in the females and appeared to depend on the correlation between bodyfat and α_2, β_1, β_2 and γ-globulins.

Table 36 shows a correlation between the E.S.R. and blood pressure, pulse frequency body temperature, hemoglobin and amounts of different types of leucocytes. A positive correlation was found between the E.S.R. and the blood pressure, and this correlation appeared to be due to age. The age factor on the other hand, does not play any rôle in the relationship between E.S.R. and number of erythrocytes. Here we are dealing with a correlation which appeared independent of the various serum protein fractions influencing the E.S.R.

A positive correlation was found between the number of leucocytes and α_2, β_1, β_2 and γ globulin, which appeared to be due mainly to a positive correlation between the serum protein fractions and the number of polynuclear leucocytes.

Table 35. *Correlations between anthropometric measurements and E.S.R., and serum proteins.*

		E.S.R.	Albumin	α_1	α_2	β_1	β_2	γ
Age	Tot	41	—.30	.07	.24	12	.24	13
	M	40	—.18	.01	.24	16	.28	.22
	F	44	—41	.17	.25	.06	.26	10
Weight		.11	—10	—.14	.05	14	.21	16
		—04	—04	—.19	.11	.21	.23	.20
		.28	—12	—.03	.00	.05	.13	18
Height		—.20	.07	—.22	—10	.02	.03	—12
		—.21	17	—.09	—.13	—.06	—.14	—13
		—.16	.21	—11	—.17	—14	—.15	—.09

As is apparent from Table 34 showing data about series A and C a negative correlation was found between albumin and various globulins. A strong positive correlation is seen between α and α_2, respectively β and β_2, and is also obvious in the total group α_2, β_1, β and γ globulins.

The E.S.R. was negatively correlated with albumin and positively correlated with various globulin fractions. These correlations could be compared with the demonstrated E.S.R. Increasing agglomerin property of haptoglobin coeruloplasmin (RUHENSTROTH BAUER et al. 1962) and 7 S-gammaglobulin (BRITTINGER 1963).

A striking negative correlation was found between the amount of albumin and age (Table 35). Of the globulin fractions α_2 and β_2 were most strongly positively correlated with age.

Table 35 also shows a correlation between the E.S.R. and bodybuild. A

Table 33 Erythrocyte sedimentation rate (mm) and electroforetic fractions of blood proteins (mg/100 ml) in Series A

		20—39 years			40—59 years			60—79 years	
		mean	SD		mean	SD		mean	SD
Males ESR	31	3.7	2.6	42	7.8	6.2	38	16.8	12.8
Alb		3.75	0.24		3.76	0.39		3.56	0.35
α		0.31	0.05		0.32	0.07		0.30	0.03
α_1		0.47	0.10		0.56	0.14		0.38	0.13
β		0.47	0.07		0.55	0.16		0.55	0.15
β_1		0.28	0.10		0.34	0.08		0.41	0.18
γ		1.12	0.16		1.12	0.28		1.23	0.19
Females ESR	34	8.8	5.2	37	16.7	10.8	28	23.3	21.8
Alb.		3.89	0.28		3.63	0.22		3.61	0.31
α_1		0.32	0.06		0.35	0.06		0.31	0.09
α_2		0.30	0.08		0.55	0.14		0.52	0.13
β_1		0.48	0.11		0.52	0.12		0.48	0.05
β_2		0.24	0.07		0.33	0.13		0.42	0.13
γ		1.12	0.23		1.27	0.20		1.15	0.29

Table 34. *Correlations between E.S R. and various electrophoreti fracti ns and between various electrophoreti fractions.*

	E.S.R.	Alb.	α_1	α_2	β_1	β_2
Alb						
Total	—.19					
Males	—.04					
Females	—.33					
α_1	.26	—.18				
	.03	.09				
	.29	—.24				
		—.39*				
α_2	.26	08	.32			
	.31	.19	23			
	27	—19	41			
		—.25	44			
β_1	.16	—14	19	46		
	.18	—.01	.29	49		
	.23	—28	.19	44		
		—.16	26	37*		
β_2	.17	—.26	13	43	.59	
	.33	—12	10	.31	60	
	.17	—.37	23	53	.67	
		—30*	.29*	23*	.39	
γ	.29	—05	08	29	.30	23
	24	01	.04	32	.28	37
	.30	—11	.07	.11	16	.33
		—.39*	45*	30*	.30*	.37*

Series C.

positive correlation with bodyfat was observed at least in the females and appeared to depend on the correlation between bodyfat and α_2, β_1, β_2 and γ-globulins.

Table 36 shows a correlation between the E.S.R. and blood pressure, pulse frequency body temperature, hemoglobin and amounts of different types of leucocytes. A positive correlation was found between the E.S.R. and the blood pressure, and this correlation appeared to be due to age. The age factor on the other hand, does not play any rôle in the relationship between E.S.R. and number of erythrocytes. Here we are dealing with a correlation which appeared independent of the various serum protein fractions influencing the E.S.R.

A positive correlation was found between the number of leucocytes and α_2, β_1, β_2 and γ-globulin, which appeared to be due mainly to a positive correlation between the serum protein fractions and the number of polynuclear leucocytes.

Table 35. *Correlati ns between anthropometri measurements, and E.S.R and serum proteins.*

		E.S.R.	Albumin	α_1	α_2	β_1	β_2	γ
Age	Tot	41	—.30	.07	.24	.13	.24	13
	M	40	—18	.01	.24	.16	.28	.22
	F	44	—41	.17	.23	.06	.28	16
Weight		11	—.10	—14	05	.15	.21	16
		—04	—04	—.19	.11	.21	.23	.20
		.18	—12	—.03	.00	.03	.13	.13
Height		—.30	.07	—.22	—.10	.03	.03	—12
		—.21	.17	—.06	—13	—.06	—.14	—13
		—18	.21	—.11	—.17	—.15	—.15	—.09

	E.S.R.	Albumin	α_1	α_2	β_1	β_2	γ
Condylar breadth	00	— 12	— 08	02	.08	.11	— .05
	— 11	— .09	— 04	00	.05	09	04
	42	— 11	12	.03	— 10	— .05	— .03
Fatfree weight	— .20	— 02	— .21	— 09	07	12	— .03
	— 18	08	.02	— 12	01	— .01	— 11
	12	.07	— .08	— 24	— 18	— 09	— .09
Bodyfat	.30	— 07	05	13	08	09	.21
	08	— 04	— 16	.20	.20	.23	.27
	.34	— .22	— 01	13	15	.30	16

Table 38. *Coefficients of correlation between blood pressure pulse frequency body temperature and number of different blood cells compared with E.S.R and various electrophoretic fractions.*

	Age	E. S. R	α_1	β_1	β_2	γ
S B P	68	.35	.34	14	.26	.32
D B P	.52	17	.21	18	17	.30
Pulse rate	11	18	10	08	— 06	16
Temperature	— 16	18	.05	00	— 04	13
Hemoglobin	— 00	— 38	— .01	12	07	00
Erythrocytes	.03	— .30	05	15	05	— 06
Leucocytes	18	04	.23	19	15	.30
Polynuclear	.20	07	.24	17	.18	.23
Mononuclear	.07	— 02	15	13	07	.03

Glutamic-pyruvic-transaminase (GPT)

The glutamic pyruvic transaminase values were higher in males (Table 37) In older age classes the values appeared to be lower especially in the males.

These observations may possibly be explained by a positive correlation between muscularity and GPT Table 38, however gives no evidence for such a correlation, which should have given a positive correlation between fatfree weight and GPT As to the elderly men, the values indicated a certain correlation between GPT and body fat.

Table 37 *Glutamic pyruvic-transaminase (GPT) in Series A.*

	20—39 years			40—59 years			60—79 years		
		mean	SD		mean	SD		mean	SD
Males	30	10.5	8.4	42	9.0	6.3	34	6.6	4.8
Females	34	6.3	6.0	37	2.7	3.1	28	4.8	3.9

Table 38. *Coefficient of correlation between GPT and bodyfat respectively fatfree weight*

		Bodyfat	Fatfree weight
Males	20—39	07	— 17
	40—59	.34	— .02
	60—79	.38	— 18
Females	20—39	.06	— .05
	40—59	.09	— 18
	60—79	16	— .26

Calcifications in arteries of lower limbs

On registration of the calcifications of the arteries in the lower limbs an attempt was made to distinguish between calcifications of the intima respectively of the media according to LINDBOM (1950). Calcifications in the media are believed to have no influence on the circulation but like other calcifications they are probably a manifestation of degenerative changes in the bloodvessel wall. Since it was not possible to make this distinction in the present investigation only 3 grades of calcification were distinguished

1. Minimal calcifications
2. Moderate calcifications
3. Marked calcifications

Such grading has been used previously in similar investigations by e.g. LANSBURY & BROWN (1934), KEIDING et al (1952) and HAIMOVICI et al (1960).

The thigh, the lower leg and the foot were judged separately.

Values obtained in the various areas were taken together after which the following classification was set up (Each of the figures represents a separate region.)

Table 39 *Distribution according to degree of calcification of arteries of lower limbs*

	Degree calc. / Age	0	1	2	3	4	5	6
Males	20—29	11	1					
	30—39	17						
	40—49	17					2	
	50—59	10	6	3	2		2	1
	60—69	5	5	3	4	2	1	3
	70—79	1	1	1	3	1	3	2
Females	20—29	12						
	30—39	21						
	40—49	16			1			
	50—59	19			1			
	60—69	13	3	2			1	
	70—79	3	1	3		1	1	

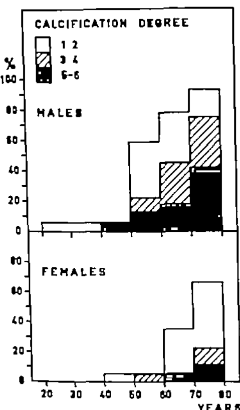


Fig 6 Degree of calcification of arteries of lower limbs at different age-levels

1 1—0—0 1—1—0
2. 1—1—1 2—0—0 2—1—0
2—2—0
3 2—1—1 2—2—1 3—0—0
3—1—0
4 3—1—1 3—2—0 2—3—1
2—2—2
5 3—2—2 3—3—1 3—3—2
6. 3—3—3

The results of these examinations are given in Table 39 and Fig 6 Fig. 6 is given as a cumulation diagram with groups 1—2, 3—4 and 5—6 taken together

It is apparent that the grade as well as the frequency of the calcifications was higher in males than in females. The extent of the calcifications in males in the 50—59 year group corresponded roughly to what was seen in women in the 70—79 year group.

On comparison between cases with the most pronounced calcifications proximally or distally in the limbs it was found that calcifications were most marked proximally in both sexes twice as often as distally

Calcifications in arteries of lower limbs

On registration of the calcifications of the arteries in the lower limbs an attempt was made to distinguish between calcifications of the intima respectively of the media according to LINDBOM (1950). Calcifications in the media are believed to have no influence on the circulation but like other calcifications they are probably a manifestation of degenerative changes in the bloodvessel wall. Since *it was not possible to make this distinction* in the present investigation only 3 grades of calcification were distinguished.

1 Minimal calcifications
2 Moderate calcifications
3 Marked calcifications

Such grading has been used previously in similar investigations by e.g. LANSBURY & BROWN (1934), KEIDING et al. (1952) and HAIMOVICI et al. (1960).

The thigh, the lower leg and the foot were judged separately.

Values obtained in the various areas *were taken together after which the* following classification was set up. (Each of the figures represents a separate region.)

Table 39. *Distribution according to degree of calcification of arteries of lower limbs.*

	Degree calc. / Age	0	1	2	3	4	5	6
Males	20—29	11	1					
	30—39	17						
	40—49	17					2	
	50—59	10	6	3	2		2	1
	60—69	5	5	3	4	2	1	3
	70—79	1	1	1	3	1	3	2
Females	20—29	12						
	30—39	21						
	40—49	15			1			
	50—59	19			1			
	60—69	13	3	3			1	
	70—79	3	1	3		1	1	

The degree of arcus lipoides corneae (a.l.c.) was judged by examination with the corneal microscope. The following symbols were used 0 = no a.l.c., 1 = slight a.l.c., 2 = moderate a.l.c., and 3 = pronounced a.l.c.

The occurrence of arcus lipoides corneae in males and females grouped according to age is given in Fig. 7. The well known correlation with age is obvious. That we found a fairly high frequency of a.l.c. (cf LINDHOLM 1960) may be due to the fact that we used a corneal microscope and therefore detected a larger proportion with only slight changes. It is clear from the curves that the more severe changes were overrepresented in males and that really severe changes were not seen in the females. The youngest person in the material with arcus lipoides corneae was a 33 year old man.

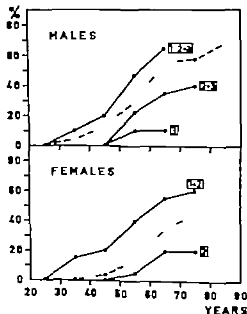


Fig. 7 Frequency of different degrees of arcus lipoides corneae at different age-levels, compared with LINDHOLM (1960)

Table 40 gives the mean intra-ocular pressure in males and females classed according to age. The pressures recorded were invariably higher in the females than in the males in corresponding age classes an observation also made by previous workers in this field (GOEDBLOED et al. 1961 STRÖMBERG 1962)

The correlation between the blood pressure and the intraocular pressure in the different age classes is given in Table 41 In the 20—39 year class no correlation could be demonstrated in males or females. In the 40—59 year class the intra-ocular pressure and the blood pressure tended to be positively intercorrelated, and in the highest age class this correlation was significant in both sexes. This correlation has apparently not been observed in earlier investigations (SIMONETT 1959)

A positive correlation with a coefficient of 0.22 was found between intra-ocular pressure and the amount of α_2-globulin in the serum which may be compared with the positive correlation between blood pressure and α_2.

Table 40 *Mean intra-ocular pressure (mm Hg) in males and females grouped according to age*

	20—39 years	40—59 years	60—79 years
Males	14.8	16 0	15 9
Females	15 6	16 4	16.3

Table 41 *Relation between blood pressure and intra-ocular pressure in males and females grouped according to age*

(— denotes persons whose intra-ocular pressure deviated from ge-corrected mean by les than 1 mm Hg.)

	20—39 years			40—59 years			60—79 years		
	Concordance	—	Discordance	Concordance	—	Discordance	Concordance	—	Discordance
Males	7	8	6	13	10	12	17	7	6
Females	15	0	18	13	22	7	17	12	7
Total	22	8	24	26	32	19	34	19	13

Arcus lipoides corneae

The degree of arcus lipoides corneae (a.l.c.) was judged by examination with the corneal microscope. The following symbols were used 0 = no a.l.c., 1 = slight a.l.c., 2 = moderate a.l.c. and 3 = pronounced a.l.c.

The occurrence of arcus lipoides corneae in males and females grouped according to age is given in Fig. 7. The well known correlation with age is obvious. That we found a fairly high frequency of a.l.c. (cf LINDHOLM 1960) may be due to the fact that we used a corneal microscope and therefore detected a larger proportion with only slight changes. It is clear from the curves that the more severe changes were overrepresented in males and that really severe changes were not seen in the females. The youngest person in the material with arcus lipoides corneae was a 33 year old man.

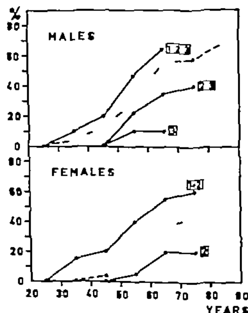


Fig. 7 Frequency of different degrees of arcus lipoides corneae at different age-levels, compared with LINDHOLM (1960)

The degree of cataract was estimated with the aid of a corneal microscope and in transmitted light with an ophthalmoscope. The following symbols were used 0=no cataract, 1=peripheral cataract not affecting vision 2=moderate cataract and 3=dense cataract.

Fig 8 shows the occurrence of cataract of varying severity in males and females classed according to age The condition was somewhat more frequent among males.

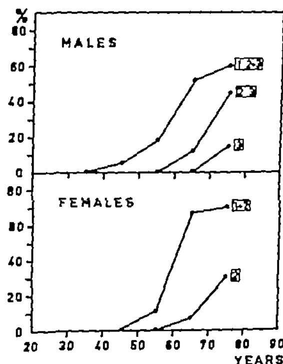


Fig 8. Frequency of different degrees of cataract at different age-levels.

Summary

An investigation was undertaken to describe the normal range of various clinical laboratory and anthropometric values as well as their inter-correlations. Special attention was given to the results of the oral glucose tolerance test.

The sample studied was derived from a population belonging to a medium sized town and a surrounding rural district. The subjects were selected according to date of birth. Of 257 persons born within the period 1884—1943 and invited to partake in the investigation, 207 co-operated.

The means and standard deviations of various data for males and females in age groups 20—39 40—59 60—79 are given.

Observations made in the investigation appeared to justify the following conclusions

1 Estimation of bodyfat by anthropometric methods gi es alues in good agreement with those found by the densitometric technique
Body weight is determined mainly

oral tolerance test are correlated with age, the correlation being closest for the 60 and 90 minute values.

4 In the higher age classes a positive correlation exists between the relative amount of bodyfat and the level of the blood sugar curve, the correlation being strongest at 60 and 90 minutes.

5 In males there is a negative correlation between the level of the glucose curve and fatfree weight, stature, and condylar breadth. The negative correlation is most marked for the 90 minute value

6. Factors varying with the blood pressure are positively correlated with the blood sugar level even after elimination of the influence of age and bodyfat.

7 Among close relatives of diabetics the fasting blood sugar level and the level during a tolerance test tend to be elevated in ages above 40 years.

8. Glycosuria following ingestion of glucose is much more common in males than in females.
Blood pressure is correlated with the amount of bodyfat. After elimination of the effect of age

on this correlation the correlation is found only in females.

10 With the exception of young women the pulse frequency is correlated positively with the amount of bodyfat and negatively with the fatfree weight

11 Body temperature is positively correlated with the amount of bodyfat

12 In males as well as in females the number of leucocytes is positively correlated with age

13 *Erythrocyte sedimentation rate* (E.S.R.) is positively correlated with various globulin fractions, *the numerical value of the cor relation* being highest for α_1 α_2 and γ globulin.

14 *The E.S.R. is negatively correlat* ed with the number of erythrocytes the correlation being inde *pendent of the correlation be* tween the E.S.R and various globulin fractions.

15 A close correlation exists between the electrophoretic subfractions α and α_{II}, β and β_{II}, and in the group α_2 β_1 β_2 and γ globulin

16 A positive covariation is demonstrable between the number of leucocytes and particularly poly nuclear cells and α_{II}, β β_{II} and γ globulin.

17 The serum glutamic pyruvic transaminase level is higher in males than in females irrespective of age and *shows a tendency to* decrease with increasing age

18 The incidence as well as the degree of calcifications in the ar teries of the *lower limbs* is substantially higher in males than in females.

19 The intra-ocular pressure seems to be higher in females than in males in all age classes.

20 *In higher age classes the intra* ocular pressure tends to be cor related with arterial blood pressure.

21 The incidence as well as the degree of arcus lipoides corneae is *higher in males than in females.*

* * *

The investigation appeared to show that in the evaluation of clinical, laboratory and anthropometric data fac tors capable of influencing such data should be taken into account particularly age

As to the oral glucose tolerance test the criteria hitherto used for the eva luation of the test do not appear to hold for old persons.

Appendix Distributions

on this correlation the correlation is found only in females.

10 With the exception of young women the pulse frequency is *correlated positively with the* amount of bodyfat and negatively with the fatfree weight

11 Body temperature is positively correlated with the amount of bodyfat

12 In males as well as in females the number of leucocytes is positively correlated with age.

13 Erythrocyte sedimentation rate (E.S.R.) is positively correlated with various globulin fractions, the numerical value of the cor*relation being highest for* α_1 α_{II} and γ *globulin.*

14 The E.S.R. is negatively correlated with the number of erythrocytes the correlation being independent of the correlation between the E.S.R. and various globulin fractions.

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* * *

The investigation appeared to show that in the evaluation of clinical laboratory and anthropometric data factors capable of influencing such data should be taken into account particularly age.

As to the oral glucose tolerance test the criteria hitherto used for the evaluation of the test do not appear to hold for old persons.

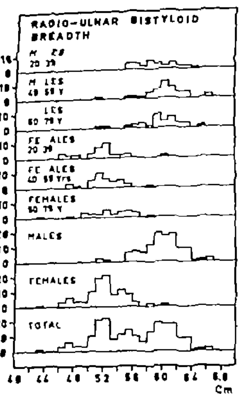


Fig. 4.

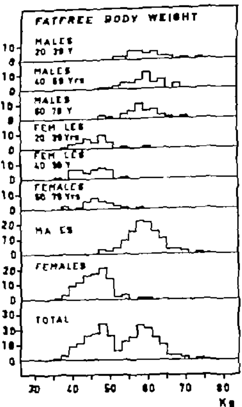


Fig. 5

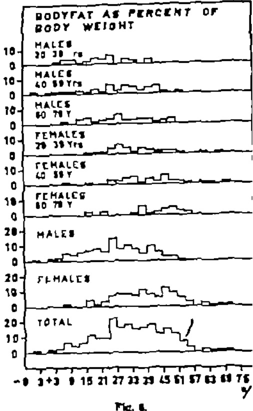


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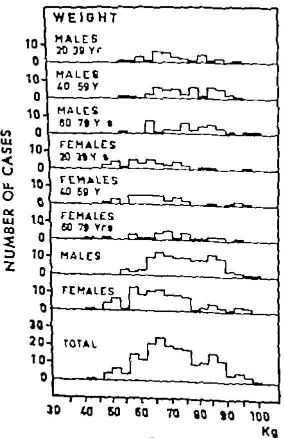


Fig. 1

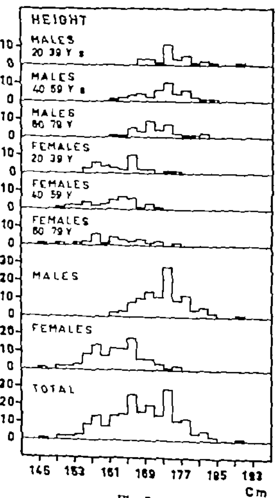


Fig 2

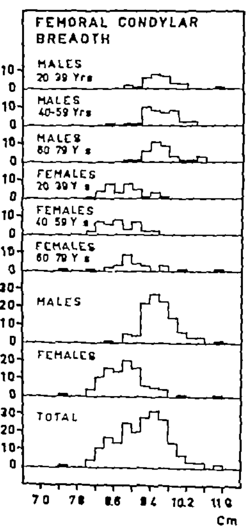


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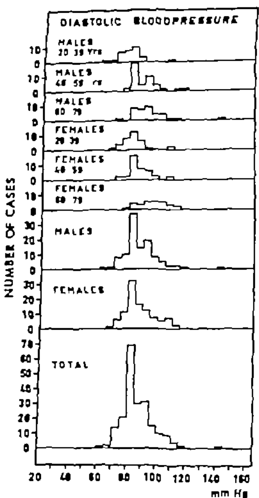


Fig. 10.

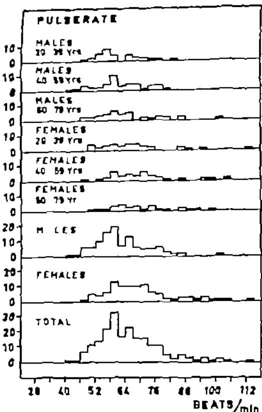


Fig. 11

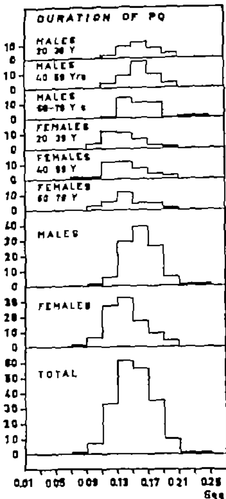


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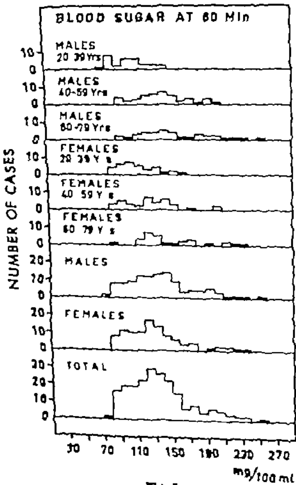


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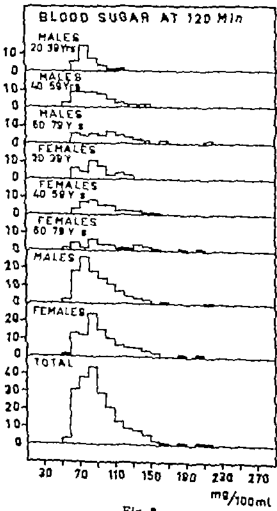


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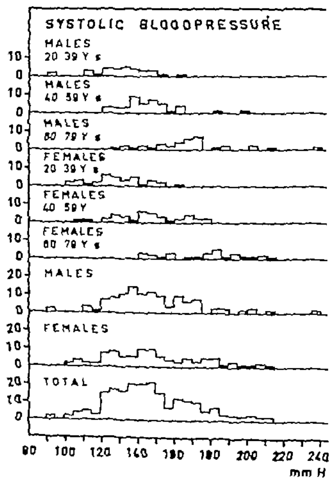


Fig. 9

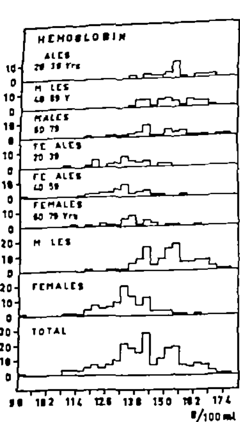


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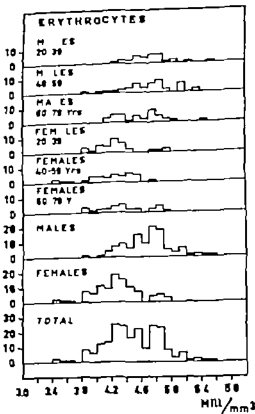


Fig. 17

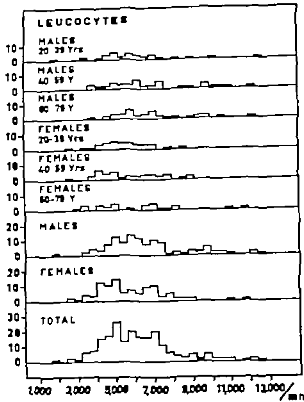


Fig 18.

NUMBER OF CASES

DURATION OF QT

MALES 70 39 Y s

MALES 40 59 Y s

MALES 60 79 Y s

FEMALES 20 39 Y s

FEMALES 40 59 Y s

FEMALES 60 78 Yrs

MA ES

FEMALES

TOTAL

0.24 0.28 0.32 0.36 0.40 0.44 0.48 Sec

Fig. 13.

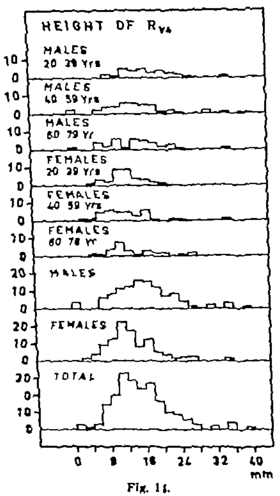


Fig. 14.

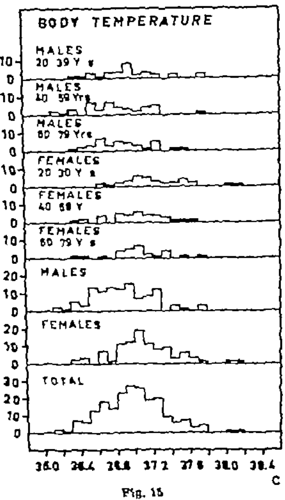


Fig. 15.

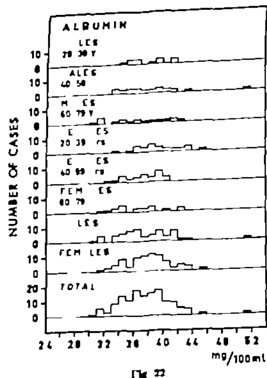


Fig. 22

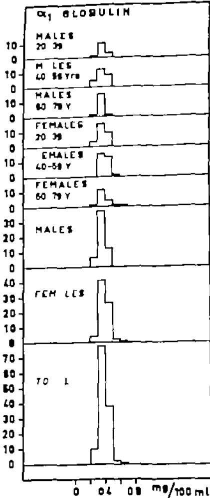


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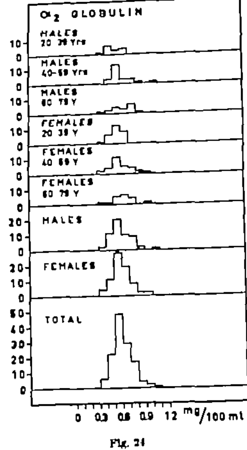


Fig. 24

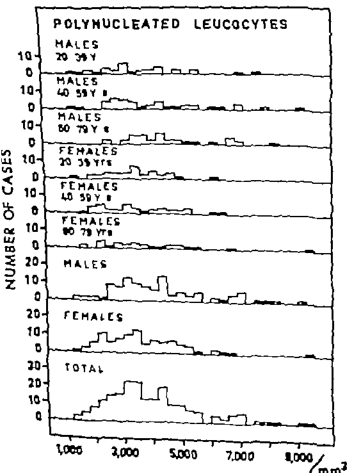


Fig 19

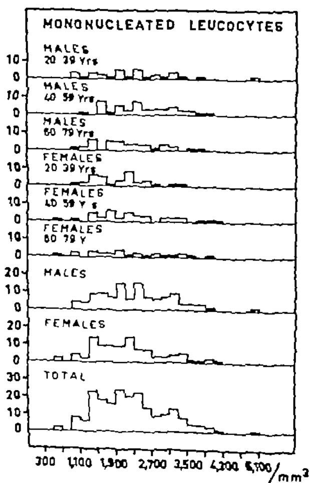


Fig. 20

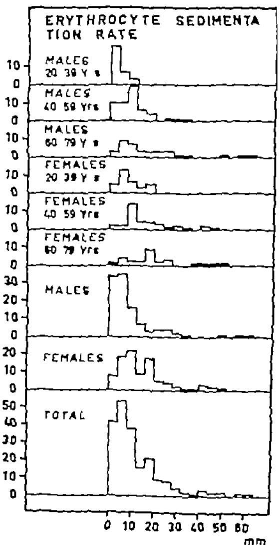
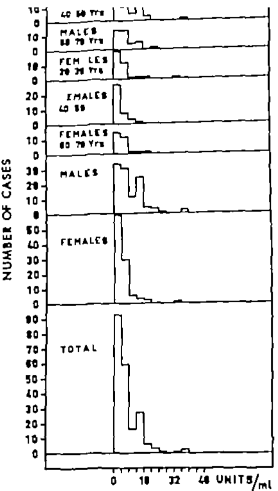


Fig. 21



NUMBER OF CASES
MALES
FEMALES
TOTAL
UNITS/ml

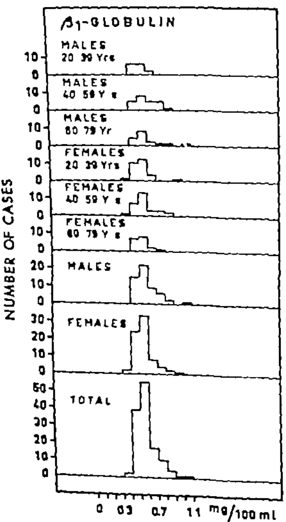


Fig 25

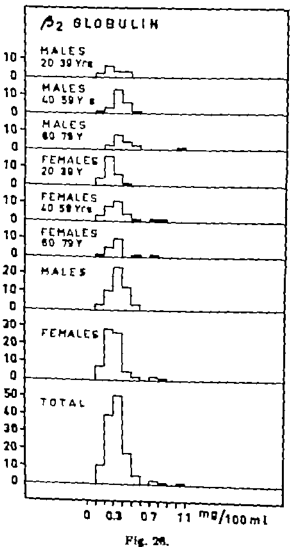


Fig. 26.

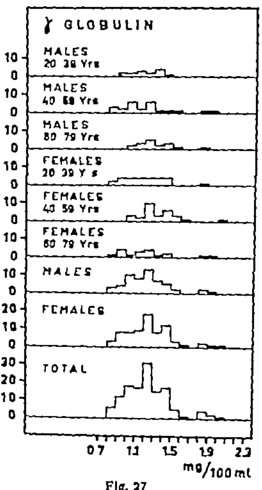
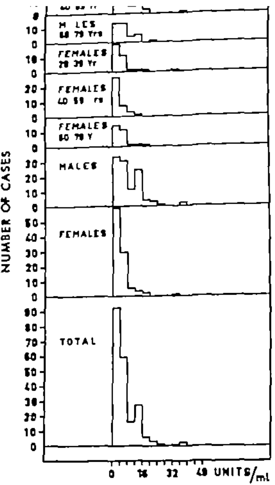


Fig. 27


NUMBER OF CASES
FEMALES
FEMALES
FEMALES
MALES
FEMALES
TOTAL
0
16
32
48
UNITS/ml

References

ANDERSON E. C. Ann. N Y Acad Sci. *110* 189 1963

AVERILL, K. H LAMB L. E. Amer J Card. 6 84 1960

BAXTER, J FORBES, G. B Ann. N Y Acad. Sci *110* 264 1963

BEAUDOIN R., VAN ITALLIE, T B MAYER J J Clin. Nutr *1* 91 1953.

BJURULF P Acta Med. Scand. suppl 349 1959

BLACKBURN H KEYS, A., SIMONSON E., RAUTAHARJU P PUNSAR, S Cirkulation *21* 1160 1960.

BRITTINGER, G Med. Klin. *58* 1037 1963.

BROZEK, J CHEN, K P., CARLSON W BRONCZYK, F Fed. Proc. *12* 21 1953

BØE, J HUMERFELT S WEDERVANG, F Acta Med Scand. suppl. 321 1957

VON DÖBELN, W Acta Med. Scand. *165* 37 1959

VON DÖBELN W Aerospace Med. 67 1961

FABER, A. Scand. Arch. f Phys. *45* 189 1924.

GOEDBLOED J., et al. Ophtalmologica *141* 481 1961

GOTTFRIED S. P PELZ, K S CLIFFORD R C. Amer J Med. Sci. 242 475 1961

HAIMOVICI, H SHAPIRO J H JACOBSON, H G. Amer J Roentgenol. *83* 1042, 1960.

HULTMAN E. Nature *183* 108, 1959

JORDE, H Acta Univers. Bergens Series Med. nr 1, 1962.

KEIDING, N R., ROOT H F MARBLE, A. J.A.M.A. *150* 964 1952.

LAURELL, C. B LAURELL, S SKOOG, N Clin. Chem. 2 99 1956.

LANSBURY J BROWN G. E. Proc Staff Meet. Mayo Clin. *9* 49 1934.

LINDBOM, Å. Acta Radiol. suppl. 80 1950.

LINDEGÅRD, B. Acta Psych. et Neurol. suppl. 86, 1953

LINDEGÅRD B Lunds Univ Årsskr N F 2, 52, Nos. 4—6, 7—8, 1956.

LINDHOLM H. Acta Med. Scand. *168* 45 1960.

MARTIN R. Lehrbuch der Anthropologie Jena, 1928

MENEELY G R., HEYSSEL, R. M., BALL, C. O. T WEILAND R. L., LORIMER, A. R., CONSTANTINIDES, C., MENEELY E. U Ann. N Y Acad. Sci. *110* 271 1963.

MOSENTHAL, H. D BARRY E. Ann. Int. Med. *33* 175 1950

NILSSON, S. E. Acta Med. Scand suppl. 375, 1962.

NILSSON S E. Acta Genet Basel, *14* 97 1964

REITMAN S E., FRANKEL, S. Am. J Clin. Pathol. 28 56, 1957

RIES, W Deutsch. Ges. Wesen *15* 991 1960.

RUDENSTROTH BAYER, G., BRITTINGER G., KAYSER, F H., NASS, G., TAUTFEST M Klin. Wschr *23* 1200 1962.

SIEVERS, J Acta Med. Scand. suppl 409, 1963

Sigma Technical Bulletin No. 505, 1960.

SILWER, H Acta Med. Scand. suppl 333, 1958.

SIMONSEN B Klin. Monatsbl Augenheilk. *135* 196, 1959

Statistical Abstracts of Sweden (Statistisk Årsbok för Sverige) 1963.

STRÖMBERG, U Acta opthalm. suppl 69 1962

TRUEDSSON, E. Acta Med Scand suppl. 381 1962

UNGER, R. H Ann. Int. Med. 47 135, 1957

WESTERGREN A. Ergebn. Inn Med u Kind. h. L. 26 677 1924

WILKERSON H L. C., BUTLER, F K FRANCIS J O. S Diabetes *9* 368, 1960.

YOUNG, C. M BLONDIN J TENSUAN R FRYER J H Ann. N Y Acad. Sci. *110* 589 1963.

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STORAGE IRON IN MAN

By

ALEKSANDER WEINFELD

ACCOMPANIES VOL. 177

GÖTEBORG 1944

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STORAGE IRON IN MAN

By

ALEKSANDER WEINFELD

GÖTEBORG 1964

Translated by
Victor Braxton

GÖTEBORG 1964
ORSTADIUS BOKTRYCKERI AKTIEBOLAG

Preface

The object of this investigation is to provide quantitative data about the state of the iron stores in the liver and bone marrow and to study their relation to other parameters of iron metabolism in the normal subject and in disease. Since the natural history in the development of iron deficiency begins with a reduction of the iron stores, interest was centered to study these interrelations in hematologically normal subjects in different states of iron repletion and to give an account of changes occurring before iron deficiency anemia ensues.

This investigation began in 1958 with histochemical studies on reticuloendothelial iron and sideroblasts and has been continued with chemical studies during the last four years. During this time I have had the privilege of working at the First Medical Department of Sahlgren's Hospital, University of Göteborg under Professor Lars Werkö, M.D. He has provided excellent working conditions, valuable support in many respects and constructive criticism for which I shall always be greatly indebted. I wish to express my deepest gratitude to Professor Jörgen Lehman, M.D. who introduced me to the field of clinical chemistry and scientific work. For facilities and aid at the Department of Clinical Chemistry during part of this investigation I am indebted to the Director of the Department, Associate Professor Gunnar Jungner, M.D. To Professor Ragnar Romanus, M.D. and to Associate Professor Yngve Edlund, M.D. and all my colleagues at the Departments of Surgery who generously helped me in gathering material for study I tender my hearty thanks. A part of the chemical work was done under the guidance of Associate Professor Søren Løvtrup, Ph.D. at his laboratory, Department of Histology, University of Göteborg and I wish to express my sincere gratitude for his support. The advice and criticism given by Assistant Professors, Bo Hallgren, M.D., Harald Hansen, M.D. and Per Lundén, M.D. are gratefully acknowledged. I wish also to express my appreciation to Professor Carl-Bertil Laurell, M.D. and Assistant Professor Lars Garby, M.D. for advice and encouragement.

Throughout this investigation the laboratory work has been skillfully and devotedly performed by Mrs Anni Jagitsh, to whom I am most grateful. My thanks are due to the following nurses and laboratory assistant, Mrs. Mary Alverborg, Mrs. Marianne Dehlin, Mrs. Ewa Johansson and Mrs. Ethel Högberg.

Advice and guidance in the statistical work has been received from Esbjörn Carlström, Ph.D. Institute of Statistics,

Translated by
Victor Braxton

GÖTEBORG 1964
ORSTADIUS BOKTRYCKERI AKTIEBOLAG

Contents

University of Göteborg his generous expenditure of time and energy is greatly appreciated

I wish to express my hearty thanks to Mr Victor Braxton for his personal engagement in translating and forming the manuscript.

This investigation has been supported by grants from the Medical Faculty of the University of Göteborg Svenska Livforsakringsbolags Nämnd för Medicinsk Forskning A. B Pharmacia, Uppsala and A. B Leo Halsingborg

Aleksander Weinfeld

To my wife and children

Contents

PART ONE

Methods for Determination of Total Non Hemin and Ferritin Iron in Tissues and Marrow Aspirates

PART ONE

Methods for Determination of Total Non Hemin and Ferritin Iron in Tissues and Marrow Aspirates

Introduction

Iron is present in tissues mainly in two forms, as hemin-bound and non-hemin bound iron. Hemoglobin, myoglobin and various iron-containing cell enzymes have the structure of hemin chromoproteins with the prosthetic iron porphyrin complex. In these compounds the iron is firmly bound, and resistent to acid hydrolysis. Non-hemin iron constitutes an iron storage fraction, present in two physically distinct protein compounds, ferritin and hemosiderin. Schmiedeberg [1] was the first to report a protein iron compound which he named ferratin. This complex which contained 6 % iron, was probably a mixture of denaturated iron protein and other proteins. In 1937 Laufberger [2] isolated an iron-rich protein from horse spleen and crystallized it with cadmium sulfate; this he called ferritin. This reddish-brown protein contained 20 % of iron by weight. Laufberger succeeded in isolating the protein through its peculiar property of being stable in aqueous solution at 80 C. When water extract of horse spleen were heated to this temperature most of the other protein were coagulated and had to be removed, while the ferritin, which remained in solution, could be crystallized with cadmium sulfate.

Kuhn *et al.* [3] confirmed Laufberger's findings, and the properties of the compound were further examined by Granick and Michaelis [4, 5]. It has been shown that the iron in ferritin possesses a characteristic magnetic susceptibility not observed in other iron compounds [6, 7].

The iron content of ferritin may range from 17 to 23 % by weight. The variable amount of iron, phosphorus and nitrogen in different ferritin preparations indicates their inhomogenous nature [5, 8]. This has been confirmed in ultracentrifuge studies by Rothen [9].

Apoferritin, an iron-free protein, is obtained when the ferric ferritin iron is reduced to the ferrous state. Apoferritin is a homogenous protein with a molecular weight of 460,000 [9]. Iron hydroxide-iron phosphate micelles of variable size and concentration are attached to apoferritin by an unknown chemical linkage and account for the inhomogeneity of ferritin [8, 9].

According to Granick [8] the iron micelles in ferritin have the approximate composition $[FeOOH]_8$ $[FeOPO_3H_2]$. The fact that ferritin fractions with different iron contents have the same Fe:P ratio confirms that phosphorus is an integral part of the micelles [10].

The molecular weight of ferritin is variable being dependent on its content of iron. In fractions of iron-rich ferritin the molecular weight has been estimated



the iron by dialysis after adding reducing agents, and crystallized apoferritin from the dialysate by adding cadmium sulphate [27]. The presence of apoferritin in aqueous extracts of iron-free hemosiderin has ben confirmed by other workers [20, 31—33].

Wöhler and Bielig [19, 20] found that the composition of the amino acids was similar for hemosiderin and ferritin from horse spleen. The fact that the similarities are not so close as between apoferritins from different species suggests the presence of other proteins [11, 32]. Wöhler's [14] report of large quantities of guanine and his view that about 25 % of the hemosiderin granules is composed of nucleic acid are contradicted by McKay and Fineberg [22].

The hypothesis of Michaelis *et al* [11] that hemosiderin may be no more than aggregated ferritin rendered insoluble has been tested recently by McKay and Fineberg [33] on the grounds that apoferritin should be solubilized on removing the iron from hemosiderin. They found that hemosiderin granules contain not only small amounts of apoferritin but also other proteins, fatty acids, cholesterol, and one or more compounds which behaved like heme. They concluded that hemosiderin is not aggregated ferritin and suggested that hemosiderin represents an iron-loaded subcellular organelle, perhaps a mitochondrion. The studies by Shoden and Sturgeon [24] have also shown that in the physico-chemical sense, "purified hemosiderin" is not a homogenous compound and that it differs distinctly from ferritin. These authors define hemosiderin in rabbit liver as an amorphous condensation of electron dense particles into an essentially protein-free aggregate, consisting mainly of ferric hydroxide, which originates in liver cell vacuoles through a process of degradation of the protein matrix of ferritin. They recommend that the term hemosiderin should be applied only when the compound under investigation is known to be insoluble in water [34].

Histochemical technics have provided evidence that inorganic compounds of iron stored in cells as hemosiderin are combined with an organic carrier containing protein, lipids and carbohydrates [35—37]. It has proved possible by electron microscopy and electron microdiffraction to distinguish hemosiderin granules, ferritin aggregations and precipitates formed after injection of colloidal iron preparations [13, 16, 29, 33—35, 38, 39]. Ferritin has been found consistently in hemosiderin granules *in situ* [38, 40, 41]. Some of them have the essential electron microscopic properties of crystalline ferritin mixed with apoferritin, others contain starch—like irregular masses in addition to irregularly arranged ferritin molecules. On the whole more ferritin was found by Richter in hemosiderin granules *in situ* than in chemically purified granules [29, 42]. He suggests that an appreciable quantity of ferritin is lost from the hemosiderin granules during the isolation procedure.

To summarize: to the chemist [34] hemosiderin is the insoluble iron-protein fraction that remains in the sediment after the tissue has been extracted with saline or water, while to the microsco-

at 800 000—860 000 Such molecules would contain 26 % of iron and the micelles would account for 44 % of the *molecular weight [11]*

In the electron microscope the ferritin molecules are readily identified as characteristic clusters of four dots, which represent iron micelles [12 13] Farrant *has shown that the molecule is com*posed of a protein shell of apoferritin and a core of inorganic ferric hydroxide [12] In more recent electron microscopic and X ray diffraction studies different models of the molecular structure of apoferritin and ferritin have been proposed *[14 15 11] All* authors now agree however that the micelles are situated inside the protein shell This explains the identical electrophoretic mobilities of ferritin and apoferritin in spite of differences in molecular weight and structure [8] and is also consistent *with the similarity of the immunochemi*cal properties of the two molecules [16—*18]*

The amino acids in ferritin and in apoferritin are identical in type and proportions [10 19—21] Kuhn [3] and Wöhler *et al* [19 20] stated that *nucleic acid is a constituent of ferritin* but this was refuted by Granick [8] and could not be substantiated *in* a recent study by MacKay and Fineberg [22]

Ferritin is relatively resistant to denaturation even at pH above 12 [10] In acid, ferritin iron goes into solution and the protein forms insoluble aggregates. If ferritin is treated with 1 *N sodium hydroxide* for 10 minutes the iron micelles are released [6] Degradation of the protein *under mild conditions* may be achieved after dehydration with *alcohol* or freeze drying Chemical and X ray crystallographic studies have shown that apoferritin splits into 18—20 subunits having a molecular weight of 25 000 [11 21]

Hemosiderin is the other iron storage compound that has long been known to *the pathologist [23] Its* golden yellow granules are visible under the microscope and they are stainable by the Prussian blue reaction The chemical composition and structure of the hemosiderin granule *is rather ill defined and variable. Hemo*siderin iron like that of ferritin, is present in the form of a colloidal iron hydroxide complex. It has a magnetic moment similar to that of ferritin although its magnitude is rather more variable [6 24] The percentage of iron *reported varies from 7 5* to 45 % *[24—*29] The composition probably depends on *the source of the hemosiderin gra*nules and on the isolation procedure Behrens and Asher who purified hemosiderin granules from horse spleen by differential centrifugation in carbon tetrachloride, concluded from their assays that the granules are probably composed of *a protein stroma*, ferric hydroxide and calcium phosphate. Studying Behrens preparations by X ray diffraction Schwietzer found that they sometimes contain crystallized forms of iron oxide identical with limonite and lepidocrocite [30] This was not confirmed in studies by Ludewig [28] Greenberg has used an inorganic suspension medium for the differential centrifugation of hemosiderin granules. From the purified hemosiderin preparations he removed

Determination of Total Non Hemin Iron in Tissue

In quantitative determinations of total storage iron in tissues it is necessary to separate the non-hemin iron fraction from the iron present in hemin compounds. The methods for effecting this separation follow three main principles.

(i) Removal of the hemoglobin from the organs by exsanguination and perfusion with saline, and determination of the iron in the hemoglobin free tissue by wet or dry ashing [43 44] By this method not only storage iron but also the residue of incompletely removed hemoglobin iron, cell hemins and myoglobin iron are determined.

(ii) Extraction of hemoglobin from tissues as hematin which is assayed colorimetrically Th s is followed by estimat on of total iron on another tissue aliquot by ash ng, and calculation of the storage iron fraction as the difference between the total and hemoglobin iron (45 46)

( ) Direct determination based on the extraction of the non-hemin iron from the tissues with hydrochloric acid (47—51) sodium pyrophosphate (52, 53) or with buffer solution, possibly with addition of reducing agents (54—56)

Whichever method is used the extraction must be complete and as little of the hemin iron as possible should be liberated The extraction with buffer solutions is incomplete unless reducing agents are added (55) on the other hand, the addition of such an agent — for instance, thioglycollic acid — releases iron from hematin (57)

The extraction procedure with sodium pyrophosphate as described by Thompsett (52) gives a low recovery but the hot sodium pyrophosphate method of Brückman and Zondek (53) yields reliable results (58)

The usual extraction medium, however has been hydrochloric acid of varying strength, hydrolysis time and temperature. The methodologic problems of the procedure have been analysed and tested by Hallgren (50)

Method

The method used in this investigation is based on that of Hallgren (50) though with some substantial modifications, by reason of which many of his checks of the method had to be repeated With 4.5 *N* hydrochloric acid in the hydrolysis tube and an extraction time of 60 minutes at 90 C Hallgren obtained complete extraction of non-hemin iron without appreciable liberation of iron from crystallized carboxy-hemoglobin of horse blood. This strength of acid would result in an undue d'lution of the sample, and this is especially undesirable

pist [37 39] it is a substance occurring in the form of intra or extra-cellular deposits that are visible under the light microscope as golden brown granules, and contain trivalent iron as demonstrated by the Prussian blue test and a variable amount of ferritin as revealed by electron microscopy

In the present investigation the term ferritin iron will be used interchangeably with water-soluble non-hemin iron"

Determination of Total Non-Hemin Iron in Tissue

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in the analysis of aspiration specimens of bone marrow and in aqueous extracts of tissues. The adjustment of the pH of the filtrate for the *o*-phenanthroline reaction by titration with ammonia and sulphuric acid, as in Hallgren's method, was inconvenient for the purpose of this study. It was found that if the re-titration with sulphuric acid is not carried out immediately after adding ammonia there is a risk of precipitating iron oxide. Thus, two modifications of Hallgren's method were made: (*i*) the extraction of non hemin iron was performed with 2.8 *N* hydrochloric acid and (*ii*) the pH of the *o*-phenanthroline reaction was adjusted to 3.0—3.2 by adding 1 M sodium citrate as a buffer.

Procedure

All apparatus and utensils are carefully washed with hydrochloric acid and thoroughly rinsed with iron free distilled water.

Fresh liver tissue, 100—1000 mg as required is homogenized with iron free water in a Potter Elvehjem glass homogenizer until an extremely fine suspension is obtained. The homogenate is transferred quantitatively to a stoppered graduated tube and made up to the required volume with water. After thoroughly mixing the suspension an aliquot of 1—4 ml is drawn from the tube and transferred to a 25 ml stoppered Pyrex tube for acid hydrolysis. After making up the volume to 4 ml with water if necessary 2 ml of 8.5 *N* hydrochloric acid is added to give a strength of 2.8 *N*. The tube is stoppered and hydrolysis is continued for 60 minutes at 90°C. After cooling, 4 ml of 20% trichloroacetic acid is added slowly to precipitate the rest of the proteins. The contents are stirred with a glass rod and allowed to stand for 20 minutes, after which they are centrifuged for 10 minutes at 3000 rev/min. The clear slightly yellowish supernatant is filtered through acid washed filter paper into a 25 ml volumetric flask. The precipitate is washed twice with 1 ml of a mixture of equal parts of 4.25 *N* hydrochloric acid and 20% trichloroacetic acid. After centrifugation the supernatants are added to the volumetric flask through the filter. Eight millilitres of 1 M sodium citrate is filtered, the paper is washed with water and discarded. One millilitre of 10 *N* ammonia is added directly to the flask, the solution is stirred and made up to 25 ml with water. The clear, sometimes faintly yellow solution should have a pH of about 3.1. From this solution two 10 ml aliquots are drawn off into separate tubes. To one of them 0.2 ml of 2% hydroquinone and 0.2 ml of 1% *o*-phenanthroline hydrochloride are added. To the other tube 0.2 ml of 2% hydroquinone and 0.2 ml of water are added. In the spectrophotometric analysis the extinction of this solution blank is subtracted from the extinction of the *o*-phenanthroline iron compound to correct for the pale yellow colour of the solution and for the slight turbidity that may occur in extracts of fatty livers, fatty bone marrow and intestine. Turbid samples with and without *o*-phenanthroline will show the same extinction when read at 600 mμ. A reagent

blank is prepared to correct for the iron content of the reagents and water. Sixteen hours after addition of o-phenanthroline the extinctions are read off in a Beckman Model B spectrophotometer at 505 mμ or in an Eppendorph spectrophotometer with filter no. 492, photocell 90B and a mercury lamp. According to the intensity of the colour developed a 10 or 40 mm cell may be used. All samples are read against water and corrected for the reagent and solution blanks.

The iron content of the sample is obtained by reading off its corrected extinction from a curve plotted for standard solutions of ferrous ammonium sulphate (Mohr's salt). (The standard solutions made from Mohr's salt did not differ by more than 1% from those made from pure iron wire.)

Comments on the colorimetric method

The technical error of the above method for standard solutions containing 1 μg of iron is 2%. With the procedure 0.5 μg of iron can be determined. An aliquot of 10—100 mg of fresh liver tissue is normally a suitable amount for analysis, but in cases of iron depletion at least 50—100 mg of tissue should be used. Smaller quantities of iron can be determined by using proportionally less of the reagents, and diluting the extract to 10 ml instead of to 25 as specified. It should be borne in mind, however, that when small specimens are analysed the biological variation will be great owing to the non-uniform distribution of storage iron in the tissue.

The o-phenanthroline reaction, with hydroquinone as a reducing agent, is influenced by the pH of the extract. As Figure 1 shows, at pH below 2.0 there is a risk of low extinction values, and at pH above 5.5 the sample may not be clear. Within the range 2.0—5.0 the extinction is fairly constant. The rate at which the colour change develops is also dependent on the pH. This variation is more marked for high iron concentrations and high pH (Fig. 2). For practically all the samples the maximum extinction was obtained 6 hours after adding the o-phenanthroline. The colour is stable for at least 50 hours.

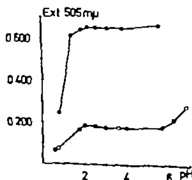


Fig. 1. Dependence of the o-phenanthroline reaction on pH. Each point is mean of 3—5 determinations.
○ 20 μg iron in sample
● 80

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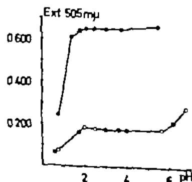


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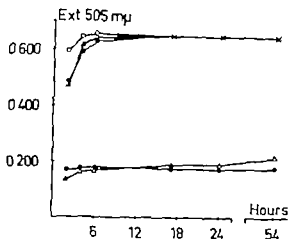


Fig 2. Influence of pH on velocity of color development in the o-phenanthroline reaction. Each point is a mean of at least 3 determinations.
O pH 2.2 ● pH 3 1
Δ 5 4—5.7 × 2.2—5 4
80 γ g iron in sample (*upper curves*)
20 " " " " (*lower curves*)

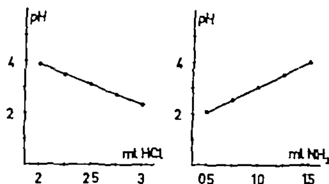


Fig. 3 Variation of pH of reagent blank with volume of 8.5 *N* hydrochloric acid (*left graph*) and 10 *N* ammonium hydroxide (*right graph*)

As Figure 3 shows, the pH of the extract remains within the range 2.0—4 0 even when the amount of the 8 5*N* hydrochloric acid is varied by ± 20% and the amount of 10 *N* ammonia by ± 50% The pH of about 3 1 obtained with the described procedure has an acceptable safety margin for the o-phenanthroline reaction

Control experiments

Control experiments were performed on 4 human livers, horse spleen and rat liver For all samples the maximum extraction of non hemin iron was obtained with hydrochloric acid strengths of 2.7 and 2 8 *N* there was no increase in yield on raising to 6 1—6 8 *N* (Table 1) This was the case for tissues with different non hemin iron concentrations and different amounts of tissue With 1 4 *N* acid the values for the human livers *were only slightly below the maximum*, but for rat livers the difference was considerable the amount of hydrolyzed tissue was three times as great as for human livers. The influence of extraction time and temperature with different strengths of hydrochloric acid was examined on 80 mg specimens of horse spleen. The amount of non hemin iron extracted was essentially the same with 2 8 *N* as with stronger acid nor was there any increase on prolonging the

TABLE 1 *Extraction of total non-haemin iron from liver and spleen with hydrochloric acid of different concentrations. Extraction time 60 minutes. Temperature 90°C. The values are means of 3—5 determinations.*

	Total non-haemin iron extracted (means, µg)					
	1.4 *N*	2.8 *N*	3.4 *N*	4.3 *N*	6.1 *N*	
Human liver						
1 (97 mg)	8.5±0.0	9.0±0.0	8.9±0.1	8.9±0.1	8.6±0.0	
2 (99 mg)	26.3±0.2	26.0±0.0	26.3±0.2	26.3±0.2	26.3±0.2	
3 (105 mg)	9.6±0.0	10.0±0.0		10.1±0.0	9.9±0.1	
4 (105 mg)		14.1±0.1		14.1±0.1	14.1±0.0	
	0.9 *N*	1.8 *N*	2.7 *N*	3.6 *N*	4.6 *N*	5.2 *N*
Rat liver						
(323 mg)	28.5	39.5	45.0	43.5	43.0	45.5
		152.0	165.0	163.0	165.0	
Horse spleen						
(120 mg)	171.0	196.0	204.0	204.0	204.0	202.0
Horse spleen						
(160 mg)	138.0	243.0	255.0	235.0	257.0	260.0
			2.8 *N*	4.3 *N*	5.7 *N*	6.8 *N*
Horse spleen						
(80 mg)			76.5±0.0	77.2±0.7	75.7±0.3	77.0±0.3

extraction time or raising the temperature (Table 2). As will be shown below the liberation of iron from the blood corpuscles increases fairly rapidly with temperature and acid strength. The absence of any change in the amount of iron extracted in these experiments was due to the relatively high concentration of non-hemin iron compared with the amount of blood present in the sample —less than 0.01 ml.

TABLE 2 *Extraction of non-hemin iron from horse spleen (80 mg samples) with hydrochloric acid. Variation of acid concentration, extraction time and temperature. The values are means of at least 3 determinations.*

		Total non-hemin iron (means, µg)		
		2.8 *N*	4.3 *N*	6.8 *N*
60 min	90°C	76.5	77.0	77.0
	120°C	76.5	75.0	76.5
120	90°C	77.0	6.5	73.3

The dependence of the efficiency of iron extraction on the amount of tissue used was examined. From a rat liver homogenate containing 100 mg of fresh tissue per millilitre, aliquots of 1, 2 and 4 ml were drawn and extracted with 2.8 *N* acid at 90 C for 60 minutes. For the samples containing 400 mg of tissue the observed values were 6% lower than the calculated ones. It is likely that larger amounts of tissue would require stronger acid or a longer time for complete extraction. That Hallgren [30] obtained complete extraction of

non hemin iron with 4.5 but not 3 6 *N* acid may have been due to less effective homogenization and the large amounts of tissue analyzed—about 1000 mg Under such conditions 4 5 *N* acid would be more effective.

The liberation of iron from red corpuscles was studied in the following experiment. Samples containing 1 ml of human blood were extracted with hydrochloric acid of different strengths and the time and temperature were also varied. In all cases slightly more iron was liberated with 1 4 than 2 8 *N* acid (Fig 4) This bears out the results reported by Venndt [59] At 90°C and an extraction time of 60 minutes approximately the same amount of iron was liberated at all strengths from 2.8 to 4 5 *N*

Treatment of many 1 ml samples of human blood with 2 8 *N* acid for 60 minutes at 90°C yielded an average of 6 9 µg of iron (range 6.5—7 0) This is

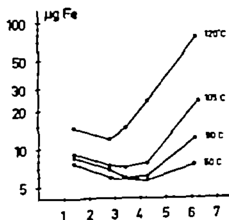


Fig 4 Variation in amount of iron liberated from 1 ml of human blood with hydrochloric acid of d fferent strengths and at different temperatures. Extraction time 60 min tes. The alues are means of 5 determinations.

equivalent to 1 4% of hemoglobin iron. Hallgren obtained 16 µg of iron from 1 ml of rat blood when using 4.5 *N* acid he reported that his values appeared to be lower for human blood [50]

If it is assumed that liver tissue contains about 8—10% of blood, and bone marrow 12% [60] then about 70 and 84 µg respectively of iron would be liberated from the hemoglobin iron per 100 g of wet tissue. Since, even in the most severe cases of iron depletion, there was not less than 500 µg of non hemin iron in 100 g of fresh liver tissue, the error in the non hemin iron determination that would be incurred by the inclusion of liberated hemoglobin iron can be regarded as negligible When however the amount of tissue and its iron content are small and the admixture of peripheral blood large the liberation of iron from hemoglobin may invalidate the results of the non-hemin iron determination This may be the case in analysis of marrow aspirates. The peripheral blood must then be removed before the acid hydrolysis is performed This problem is discussed below (p 33)

The technical error of the method for various amounts of iron in the analysed sample of liver tissue is shown in Table 3 The mean standard error of a single determination calculated from a random 102 duplicate determinations was ±0 16, or 1.5% and the error of the mean of a duplicate determination was 1 1%

The method of extracting non-hemin iron with 2 8 *N* hydrochloric acid at 90 was examined by recovery experiments. To aliquots of rat liver homogenate the non hemin iron content of

TABLE 3. *Technical error of the method of total non-hemin iron determination calculated from 102 duplicate determinations on liver biopsy specimens.*

Iron content of liver sample: range (µg)	n*	Standard error of single determination ±µg	%	Standard error of the mean of duplicate determination %
0—2	12	0.1	5.5	3.9
2.1—5.0	30	0.1	2.8	2.0
5.1—10.0	30	0.1	1.8	1.3
> 10.0	30	0.2	1.0	0.7
Total				
0—47	102	0.16	1.5	1.1

* Number of liver biopsy specimens on which duplicate determinations were done.
The standard error of a single determination was calculated according to the formula $\sqrt{\Sigma d^2/2}$

TABLE 4 *Recovery experiment (1). Mohr's salt added to samples of rat liver homogenate Extraction of non-hemin iron with 2.8 N hydrochloric acid Extraction time 60 minutes, temperature 90°C.*

Initial total non-hemin iron in liver sample* (µg)	Iron added as Mohr's salt (µg)	Total iron recovered (µg)	Added iron recovered (µg)	Added iron recovered (%)	
9.9±0.2	5	14.5	4.5	90	96
		15.5	5.1	102	
	10	20.5	10.6	106	104
		20.0	10.1	101	
	20	28.8	18.9	95	97
		29.5	19.6	98	
	30	39.5	29.6	99	98
		38.7	28.8	96	
	40	48.8	38.9	97	101
		52.0	42.1	105	
	10	19.5	9.6	96	98
		20.5	10.6	106	
		19.5	9.6	96	
		20.0	10.1	101	
		19.0	9.1	91	

* Mean of 7 determinations

which had already been determined on many samples different amounts of Mohr's salt were added and the total iron extractable with acid was determined (Table 4) In a second experiment solutions of crystallized horse ferritin were added to horse spleen and rat liver homogenates with known contents of non-hemin iron. The recovery of the added iron salt and ferritin iron ranged in both experiments from 90 to 106 % (Table 4 and 5)

TABLE 5 *Recovery experiment 2. Crystalline horse ferritin added to samples of horse spleen and rat liver Extraction of total non-hemin iron as in Recovery experiment 1*

	Initial total non-hemin iron in sample (µg)	Ferritin iron added (µg)	Total non-hemin iron recovered (µg)	Added ferritin iron recovered (µg)	(%)
Horse spleen (48 mg)	40.2±0.3	16.6±0..	57.8±0.3	17 6±0.3	106
Rat liver (140 mg)	24 0	25.5±0 0	48.5	24.5	96

Determination of Ferritin Iron

In certain studies it is not only the total amount of storage iron present that is of interest but also the proportion of its two colloidal forms, ferritin and hemosiderin. Three methods are described for the determination of ferritin iron in tissues. All of them are based on two properties of ferritin, namely its solubility in water (in contrast to hemosiderin) and its stability at 75—80 C (other proteins are removed by coagulation at this temperature)

The quantitative immunochemical method of Mazur and Shorr [16] is a hihighly specific one, but because of its complexity it is used only in special work. The method of Keiderling and Wöhler [61] is based on extraction of ferritin with water removal of other proteins by heating and filtration, and subsequent electrophoresis of a condensed solution. The iron or protein content is determined in a densitometer after staining the strip

The method of Gabrio *et al.* [62] is the most simple one. The tissue is extracted twice with water and centrifuged at 1400 g The supernatants contain ferritin and hemoglobin The hemoglobin concentration is determined by a hemochromogen method and the total iron content of the extract is assayed after digestion The ferritin iron is then obtained as the difference between the hemoglobin iron and the total iron in the extract. The hemoglobin may also be removed by heating the extract to 75 C. The residual sediment of the extracted homogenate contains the hemosiderin iron fraction, which may also be determined after digestion.

Method

The method used in this investigation is based on the fractionation procedure employed by Gabrio *et al.* but differs from it in two respects: (*i*) the non-hemin iron is determined directly by acid hydrolysis, as described above, so that it is not necessary to correct for hemoglobin iron (*ii*) the aqueous extracts, whether they are heated to 75 C or not, are always centrifuged finally at 19,000 to 20,000 g for 60 minutes before the acid extraction of non-hemin iron is performed. The latter modification was introduced because clear extracts could not be obtained consistently by conventional centrifugation at 1400 or 2000 g — either with or without heating to 75 C. Furthermore, solid brown sediments appeared on standing or on recentrifugation at the same or higher speed. The iron concentration in the supernatants diminished after the second centrifugation. These differences were

most pronounced when the *extract was* derived from a tissue rich in hemosiderin or there was much sediment after re-centrifugation. Although duplicate determinations of non hemin iron in extracts obtained with a given centrifugal force gave consistent results it is not justifiable *to consider cloudy samples* as constituting the water soluble iron fraction of the tissue. The presence of sediment and the reduction in the iron concentration of the supernatant observed on renewed centrifugation indicate that water in*soluble* hemosiderin particles were probably *suspended* in the extract (Table 6) Gabrio *et al* [62] *found that by* varying the speed of centrifugation it is possible to change the proportion of the hemosiderin and ferritin fraction and they postulated the existence of a graded series of molecular aggregates of ferritin. It might thus be inferred that when a *high centrifugal force* is used part of the water soluble *ferritin* can be lost. This and associated problems were examined experimentally

TABLE 6. *Comparison between the non-hemin iron content of water extracts of rat liver homogenates prepared by centrifugation at 1400 g and without heating (A) and extracts heated t 75°C and centrifuged at 20,000 g (B).*

Human liver	1	2	3	4	5	6	7	8	9	10
Water extract (mg/100 g w wt)										
A 1400 *g*	26.0	24.8	28.2	28.0	13.5	18.4	19.3	21 6	13.4	29 4
B 20 000 *g* and heating to 75 C	13 1	17 6	19 1	18.8	7 6	7 9	13.0	16.3	9.5	20.3
Total non-hemin iron (mg/100 g w wt)	30.8	33.3	33.5	37 0	18.1	23.0	23.4	27 0	20.9	42.3

Procedure

The tissue is homogenized with iron-free distilled water adjusted with sodium hydroxide to pH 7 Two millilitres of the fine homogenate containing about 50—200 mg of liver tissue are transferred to a *stoppered 4 ml microcentrifuge* tube, placed on a mechanical blood mixing apparatus for 60 minutes and centrifuged in a swing-out head at 1400 *g* for 20 minutes. The supernatant is transferred to a *stoppered 5 or 10 ml* graduated cylinder One millilitre of water is added to the sediment in the microcentrifuge tube and the extraction is continued for 10 minutes on the mixer apparatus, followed by centrifugation at *1400 g for 20 minutes. The* supernatant is added to the former in the graduated cylinder The extraction is repeated once more in the same way

The combined supernatants are usually made up to 5 ml with water If the iron concentration in this volume is suspected of being higher than 5 μg/ml it is diluted correspondingly This extract is usually cloudy and contains a fine suspension of tissue particles. It is then placed in a water bath at about 55°C and the temperature is raised to 75° at which

level the extract is heated for 5 minutes and stirred with a glass rod. The extract is then cooled with cold water and transferred to a polypropylene tube. The final centrifugation is performed at 20,000 g (R_{max}) for 60 minutes in a "High-Speed 17" MSE centrifuge (angle head no. 69182, with a radius of 107 mm to the inside tip of the tube). From the clear supernatant an aliquot of 4 ml is drawn for analysis of non-hemin iron.

Comments

Reddish extracts usually contain hemolyzed blood. After heating and centrifugation such samples are pale owing to coagulation and removal of the hemoglobin together with other proteins. Although the liberation of iron from hemoglobin by the acid hydrolysis is usually negligible it may be significant when the iron content of the sample is low and the amount of blood present relatively high. The determination of non-hemin iron in heated samples would then be more reliable.

Some extracts are not clarified by high-speed centrifugation alone but only in combination with heating. Even then, slight turbidity may persist. This occurs in a small number of cases, for instance samples of fatty livers, bone marrow or intestine, when it is due to the high content of neutral fat. It is then essential to correct the extinction of the colour reaction as detailed above (p. 14).

Water extracts from liver tissue are, however, usually clear after the final centrifugation and heating is therefore unnecessary. As will be shown below the difference in iron content between heated and unheated extracts is negligible.

Control experiments

For accurate tissue fractionation into water insoluble and water-soluble iron all the latter should be extracted, there should be no insoluble iron, and no soluble iron should be lost in the subsequent processing.

COMPLETENESS OF EXTRACTION.—From 4 homogenates of human liver each containing a different amount of iron, aliquots were transferred to microcentrifuge tubes for extraction with water. They were extracted once, twice, three or four times and their respective supernatants combined. After dilution to volume all were centrifuged at 20,000 g for 60 minutes and the non-hemin iron was then determined. Apart from the variation of the number of extractions the procedure was as given above.

No further water-soluble iron was removed after the second extraction, even from samples containing fairly large amounts of iron, and with a tissue-to-water ratio of 1:4 at extraction (Table 7). This is in agreement with the results reported by other authors [61—3]. For reasons described below greater dilutions were preferred, and a ratio of 1:20 was usually employed.

EXTRACTION WITH DIFFERENT MEDIA.—According to Granick [8] ferritin crystals have a low solubility in water but dissolve when salted in with 2% ammonium sulphate. The ferritin present in tissues, however, seems to be as soluble

TABLE 7 *Water-soluble non-hemin iron obtained with 1—4 extractions. The values are means of 3 determinations*

Liver specimen (mg)		Ratio of tissue weight to water	Water-soluble non-hemin iron extracted			
			1 extr	2 extr	3 extr	4 extr.
A	100	1 19	12.7±0 4	13 9±0 1	14 0±0.1	14.1±0.1
B	200	1·9	7 6	8 6±0.1	8.5±0.1	8.5±0
C	200	1·9		29 8±0.4	29 1±0.4	29 0±0.3
D	400	1·4		54.2±0.2	55 0±0	54.3±0.3

in water as in 2% ammonium sulphate [42] Shoden and Richter suggest that the concentration of salts in the tissue is high enough for the extraction of ferritin In the following experiment the efficiency of extraction of ferritin with various media was examined.

Five aliquots of about 1.5 g of liver tissue were homogenized in water at pH 7 0 9 % saline, 1/15 M phosphate buffer solution in 0 9% saline, 3.7 % aqueous sodium citrate and 1 M sodium bicarbonate. The total non-hemin iron concentration of the respective homogenates was determined in duplicate, and the soluble ferritin iron was extracted with the respective media by the above procedure The tissue-to-liquid ratio was 1·9 The percentage of extracted ferritin iron in relation to the total non hemin iron content was essentially the same in the distilled water extracts as in 0 9 % saline extracts (Table 8) the phosphate extraction gave slightly lower values and the citrate slightly higher ones but the differences from the saline value were not much greater than the variation observed when slices from different parts of the liver were extracted with the same medium The values for the bicarbonate, however were appreciably lower

SEDIMENTATION OF FERRITIN — Apoferritin is a homogeneous, monomolecularly dispersed protein with a well defined sedimentation constant of 17.6 Svedberg units [9] Ferritin solution, on the other hand is heterogeneous the particles of apoferritin—iron complex

TABLE 8 *Ext act on f the sol bl non-hemin ron f action with various media. The values are means of 2 o 3 determ nat ons.*

Extraction medium	Total non-hemin iron in homogenate (mg/100 g w wt)	Non-hemin iron in extract (mg/100 g w wt)	(%)
Water (pH7)	35 7±0 7	21.5±0.2	60
Saline 0 9 % (pH7)	34 6±0.0	21 6±0.3	62
M phosphate in 0 9 % saline (pH7 5)	33 4±0 4	19.2±0.1	58
3.7 sodium citrate	33.9±0 0	22.5±0.8	66
1 M sodium bicarbonate	31 9±0.4	15.5±0 9	49

are not uniform in size, and are much larger than apoferritin, with an average sedimentation constant of 65 Svedberg units [9]. After ultracentrifugation of the brown ferritin solution a continuous spectrum from pale yellow to dark brown may be seen in the tube [15]. Because of the polydisperse nature of ferritin and the possibility that it may form aggregates of particles, its behavior at more conventional centrifugal forces was examined.

Crystalline ferritin from horse spleen and human liver was prepared by the methods of Mazur & Shorr [18] and Mende & Sunderman [64][1]. Aqueous and saline (0.9 %) solutions of ferritin with a range of concentrations were prepared and centrifuged for one or 2 hours at different speeds. An MSE High-Speed 17 centrifuge with the angle head no. 69182 and the Spinco L Ultracentrifuge, Roto no. 40 were used. The centrifugal force was calculated on the basis of R_{max}, that is, the distance from the axis to the bottom of the tube. Tubes of semitranslucent polypropylene or nitrocellulose were used. After centrifugation the color of the various layers of the tube was examined, care being taken not to agitate the fluid. Aliquots were drawn from different layers of the tube for assays of non-hemin iron. The aperture of the pipette was always kept near the surface of the liquid.

With high ferritin concentrations (above 50 μg of iron per millilitre of solution) there was a slight tendency for the iron concentration to diminish in the upper layer at only 2900 *g* (Table 9). At 12,000 *g* a darker brown colour was observed at the bottom of the tube. This became increasingly pronounced as the speed was raised: at 26,000 *g* it was a strong brownish red, and diminished gradually in intensity towards the surface; there was a corresponding decrease in the iron concentration. The weaker ferritin solutions, both aqueous and saline, with 10 μg of iron per millilitre or less showed no tendency to sediment after 2 hours of centrifugation at 26 000 *g*. Solutions with 15 μg of iron showed a slight tendency to sediment at 26,000 *g* and weaker solutions with 5 μg at 36,000 *g* in the Spinco L Ultracentrifuge.

Since the iron content of the tissue extracts was never as high as 10 μg/ml after dilution and was usually lower than 5 μg, there was no liklihood that ferritin would be lost from the solution at a force of only 20,000 *g*. Native ferritin solutions from tissue extracts probably tend to aggregate less than purified crystalline ferritin, as is suggested by the results in Table 12.

It must be emphasized that when concentrated extracts are used there is a risk that ferritin may be lost by sedimentation even at relatively low centrifugal forces. This is one reason why dilute extracts were used in the present study.

Heating of water extracts of liver tissue. — According to Granick [8] dilute solutions of ferritin and apoferritin remain clear when heated to

[1] I wish to acknowledge my indebtedness to Civ. Ing. Sture Lindvall, at the Astra Laboratories, Södertälje, for preparing and purifying the ferritin.

Table 9 *Concentration of non-hemin iron in purified crystalline horse ferritin solutions before and after centrifugation at different forces The values are means of 3 determinations.*

Medium	Concentration of non-hemin iron (μg/ml solution)					
	No centrifugation	Centrifugation RCF (R_{max})		The layer of the solution		
		g	min.	Top	Middle	Bottom
		MSE CENTRIFUGE				
Saline 0.9 %	53.5±0.8	2900	60	50.0±0.0		52.5±0.0
	56.8±0.4	12,000		53.1±0.2	53.3±1.2	55.5
Distilled water	5.0±0.1	26,000	120	5.0±0.1		
	10.1±0.1			10.3±0.1		9.4±0.6
	10.1±0.2		60	9.9±0.1	9.9±0.1	9.8
	15.4±0.0			14.3±0.1	14.4±0.2	15.3±0.1
Saline 0.9 %	6.0±0.1		120	6.1±0.0		
	9.6±0.1		60	9.6±0.1	9.4±0.1	9.2±0.1
	15.4±0.0			14.1±0.1	14.8±0.3	15.5±0.0
	56.0±0.0			45.8±0.2	61.0±0.0	64.5
	56.8±0.4		120	46.0±1.3	50.1±2.8	94.5
		SPINCO L CENTRIFUGE				
Distilled water	5.0±0.1	36,000	60	4.4±0.1		4.6±0.0
	10.1±0.1			6.6±0.5		14.6±1.4
Saline 0.9 %	6.0±0.1			4.1±0.0		7.0
Distilled water	9.7±0.0	56,000	60	2.1±0.1		19.3±1.6
Saline 0.9 %	15.4±0.0			5.5±0.0		21.0
	10.0±0.0			2.2±0.1		18.2±1.0
	15.4±0.0			0.5		29.3

80°C, whereas concentrated solutions of 2% and above become cloudy at 65—70° but clear on cooling The stability of ferritin at 80 has been made use of by Gabrio *et al* [62] for fractionation of water extracts into hemoglobin and ferritin. With a radioisotope technique these authors found a loss of 6% of ferritin to the heat coagulum Their immunochemical measurements indicated, however a more complete recovery

From 27 homogenates of surgical liver specimens water extracts were prepared without and with heating for 5 minutes at 75° The procedure was otherwise as described above.

The values for heated extracts were on an average 2.9% lower (statistically significant) The slight differences which occurred in only one-half of the samples may be due in part to the removal of hemoglobin iron by heating and not only to loss of ferritin iron

Filtration of ferritin — Attempts to remove impurities by filtering through filter paper as has been done by other workers [61] gave disappointing results the cloudy extracts did not clear after filtration and still gave a solid sediment after recentrifugation. Furthermore, after filtration of clear tissue extracts and

pure ferritin solutions a drop in the iron concentration was observed on several occasions. The results were not consistent, however and the following two experiments were therefore carried out.

Aqueous and saline (0.9%) solutions of crystalline horse ferritin were adjusted with buffers to different pH and passed once or twice through filter paper (Munktell OOH). The acid washed filter papers were thoroughly washed with water at pH 7 and dried before use. The non-hemin iron concentrations were determined before and after filtration. During filtration there was an extremely sharp fall in the iron concentration, most pronounced for the solutions of low pH. This indicates adsorption of ferritin iron by the paper (Fig. 5).

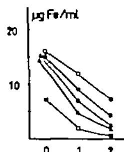


Fig. 5. Concentration of non-hemin iron before and after one (1) and two (2) filtrations.

Ferritin in aqueous solution
○ pH 7 (fosfate buffer) ● pH 6 (citrate — NaOH buffer)
△ pH 5 (citrate-NaOH buffer) ▲ pH 4 (citrate — HCl buffer)
Ferritin in 0.9% saline
□ pH 6 (citrate — NaOH buffer)

In the second experiment liver tissue was extracted with water, saline, fosfate, citrate and bicarbonate. After the final centrifugation at 20,000 g all the extracts were clear. The concentration of non-hemin iron was determined before and after one, 2 and 3 filtrations (Munktell OOH). At these iron concentrations and a pH range of 6.5 to 8.5 there was no loss of iron (Table 10). No systematic experiments were done at lower pH. However on several occasions a decrease in iron concentration was observed after filtration of extracts with a moderately high iron concentration. As a result of these findings the filtration of tissue extracts in the quantitative determination of ferritin iron was abandoned.

TABLE 10. *Concentration of non-hemin iron in liver tissue extracts with various media before and after filtration through filter paper. Means of 3 determinations.*

	pH	Non-hemin iron (µg/ml solution) No filtration	1 filtration	2 filtrations	3 filtrations
Distilled water	6.8	11.0±0.1	10.6±0.2	10.8±0.2	10.2±0.0
Saline 0.9%	6.5	7.4±0.1	7.6±0.1	7.0±0.0	6.8±0.0
Fosfate buffer in 0.9% saline	7.3	7.0±0.1	6.8±0.1	6.8±0.1	7.0±0.0
3.7% sodium citrate	7.6	9.8±0.2	9.4±0.1	9.2±0.1	9.4±0.1
1 M sodium bicarbonate	8.4	8.8±0.0	8.6±0.1	8.8±0.0	8.8±0.1

Recovery experiments

Four recovery experiments were performed

(*i*) Ammonium ferrous sulphate was added to homogenates of 3 human livers From these homogenates 2 ml samples containing 80 mg of tissue were drawn transferred to microcentrifuge tubes and extracted three times as described under *Procedure* The recovery of the iron salt ranged from 87 to 92% with a mean of 89% (Table 11) This rather low value may possibly be ascribed to the presence of iron-binding proteins that either are insoluble in water or were removed through coagulation on heating.

TABLE 11 *Recovery of iron from aqueous extracts of liver tissue to which ferrous ammonium sulphate had been added The values are means of 3 determinations*

Initial non-hemin iron (μg)	Iron added (Mohr's salt) (μg)	Total iron found (μg)	Added iron recovered (μg)	(%)	
0.48 ± 0.11	3 8 ± 0.0	3.9 ± 0.07	3.4	89	
0.48 ± 0 11	6.4 ± 0 0	6.4 ± 0 07	5.9	92	89
0.48 ± 0.11	12.8 ± 0 0	11.6 ± 0.26	11 1	87	

(*ii*) Recovery of iron from added crystalline horse ferritin solution Horse spleen tissue was extracted with water and centrifuged at 1400 *g* From this turbid extract aliquots were transferred to two volumetric flasks To one of them ferritin solution of a known iron concentration was added From both flasks aliquots of 1 ml were drawn and processed in various ways, as indicated in Table 12

The recovery of added ferritin iron ranged from 94 to 101% with a mean of 96% These was no significant difference in ferritin iron recovery for the heated and unheated samples. In spite

TABLE 12. *Recovery of iron added as crystalline horse ferritin sol tion to cl dy aq eous extracts of horse spleen. The value are means of 3—5 determ nations.*

Processing of the cloudy extract of horse spleen	Initial non-hemin iron (μg)*	Horse ferritin iron added (μg)*	Non-hemin iron found (μg)*	Added ferritin iron recovered (μg)*	(%)	
None	13 7 ± 0 5	16.9 ± 0 2	30 0 ± 0 0	16.3	96	
Heating at 75 C, centrifugation at 1400 *g*	10 6 ± 0.2		6.8 0.3	16.2	96	
Final centrifugation at 20 000 *g*	10.1 ± 0.2		26.0 ± 0 0	15 9	94	96
Heating at 75 C, final centrifugation t 20 000 *g*	9 7 ± 0 5		25.5 ± 1.3	15.8	94	
Centrifugation at 20,000 *g* heating at 75 C, final centrifugation at 1400 *g*	9 7 ± 0.3		26.9 ± 0.2	17...	101	

* Per millilitre of the processed extract.

of the fairly high concentration of ferritin iron in those experiments there was no difference in recovery in samples processed by centrifugation at 20,000 and 1400 g (Table 12)

(iii) Recovery of iron from human crystalline ferritin solution added to human spleen. Iron-depleted spleen tissue was homogenized and 4 aliquots were drawn and transferred to separate volumetric flasks. To 3 of them different amounts of ferritin were added. The same amounts of ferritin were placed in similar flasks without homogenate. From all the flasks 2 series of 2—5 aliquots of 2 ml were drawn into microcentrifuge tubes and processed without and with heating, as indicated in Table 13 The mean recoveries of ferritin iron were 95% and 99% respectively with a range of 92—100% (Table 13)

TABLE 13. *Recovery of iron added as crystalline (human) ferritin solution to human spleen homogenates The values are means of 2—5 determinations.*

Processing of the sample with spleen homogenate	Initial non hemin iron in tissue aliquot (µg)*	Ferritin iron added (µg)*	Non-hemin iron found (µg)*	Added ferritin iron recovered (µg)	Added ferritin iron recovered (%)	
Three extractions with water; final centrifugation at 26,000 g	0.15 ± 0.05	3.7 ± 0.2	3.8 ± 0.0	3.7	100	95
		8.0 ± 0.1	7.5 ± 0.1	7.4	93	
			9.3 ± 0.0	9.2	92	
Three extractions with water; heating at 75° C, final centrifugation at 26,000 g	0.15 ± 0.05	3.7 ± 0.1	3.8 ± 0.0	3.7	100	99
		7.8 ± 0.1	7.9 ± 0.0	7.8	100	
		10.0 ± 0.0	9.7 ± 0.1	9.6	96	
No extraction. Direct acid hydrolysis for non-hemin iron determination	0.4 ± 0.07	4.0 ± 0.0	4.6 ± 0.0	4.2	105	
		8.0 ± 0.0	8.6 ± 0.0	8.2	103	
		10.0 ± 0.0	10.8 ± 0.0	10.4	104	

* Per 4 milliliters of the processed extract.

(iv) Crystalline horse ferritin solution was added to horse spleen tissue: the procedure was as for the former experiment and as indicated in Table 14 The mean recovery of ferritin iron was 99% with a range of 95—106%

In neither (iii) nor (iv) was there any appreciable difference in recovery for heated versus unheated extracts and for 1400 versus 20,000 g centrifugation. In the experiment with human ferritin (Table 13) in which low ferritin concentrations were used, the mean recovery was 96% even though a force of 26,000 g was applied in the final centrifugation.

In experiments (iii) and (iv) the total error of the tissue fractionation is included.

Table 14 Recovery if iron added as crystalline horse ferritin solution to horse spleen homogenate The values are means of 3—5 determinations.

Processing of the sample with horse spleen homogenate	Initial non-hemin iron (µg)	Horse ferritin iron added (µg)	Non-hemin iron found (µg)	Added ferritin iron recovered (µg)	(%)	
Three extractions with water centrifugation at 1400 *g*	*10 6±0 42*	*8.3±0 11*	*19 4±0.63*	*8.8*	*106*	
	13.3±0.29	8.3±0 11	21.2±0.29	7 9	95	99
Three extractions with water heating at 75 C, *final centrifugation* at 20 000 *g*	10.2±0.45	8.3±0.11	18.1±0.42	7 9	95	

Error of the method

The technical error of the method was determined from duplicate determinations on heated samples of 55 human livers. The standard error of a single determination was 1 9% the range of the ferritin iron concentration was 0 6—53.2 mg of Fe/100 g of tissue, *with a mean of 10 6 mg*

Fractionation of liver tissue

A weighed piece of fresh liver tissue is homogenized with iron free water at pH 7 (6 9—7 1) in a 5 ml Potter Elvehjem all-glass homogenizer driven by a variable-speed manually adjusted electric stirrer motor The most suitable weight of the specimen is 300—500 mg if it is not intended to perform the full analysis program 50—100 mg will suffice. The fine homogenate is transferred to a 10—15 ml graduated cylinder provided with a stopper Washings from the homogenizer are added to the cylinder and water is added to the desired volume. In this study the ratio of the weight of fresh tissue to the volume of liquid ranged from 1 10 to 1.20 The homogenate is then mixed thoroughly and allowed to stand for 20—30 minutes. Before each aliquot is to be drawn the cylinder is inverted 30 times. One or two aliquots of 1 ml are drawn for determination of the dry weight, for which at least 30 mg of fresh liver tissue is required. The specimen is dried over phosphorus pentoxide and then to constant weight in an oven for 12—24 hours at 80 Alterna tively the samples may be freeze-dried to constant weight.

Two aliquots of 2—4 ml are transferred to 25 ml stoppered Pyrex tubes for acid hydrolysis and determination of the total non hemin iron as described above Two aliquots of 2 or 3 ml are transferred to 4 ml conical stoppered Pyrex microcentrifuge tubes, for preparation of water extracts.

A New Method for Quantitative Determination of Non-Hemin Iron in Bone Marrow Aspirates

There are few reports dealing with quantitative determinations of non-hemin iron in bone marrow in general and in aspiration biopsies in particular. Hallgren [50] was the first to determine the non-hemin iron content of human marrow on autopsy material and to compare it with the iron content in liver and other organs. He has also done some determinations on saline washed biopsy specimens of bone marrow from iron-deficient subjects. A similar technic was used by Stenninger [65]. Kerr [66] has determined non-hemin iron from bone marrow aspirates washed in saline and used protein as a basis for the iron determination.

The paucity of reports regarding non-hemin iron determinations in bone marrow aspirates is astonishing in view of the fact that the marrow is the most easily accessible site of examination *in vivo* and besides, of special interest for hematological work. The reason probably lies in the methodologic difficulties.

General considerations

Since there is no practical method at present available for determining the total amount of storage iron in the bone marrow our determinations must rely upon a relatively small sample drawn from part of the organ, on the assumption either that it is representative of the whole or that there is a systematic relationship between the region examined and rest of the organ. Such an assumption is in some measure justified since the differential count of the nucleated marrow cells is similar for red marrow at different sites [67—69]. It remains to be shown, however that this is true also of non-hemin iron.

Non-hemin iron is located chiefly in the red marrow and follows the reticulum cell in its distribution. The yellow marrow contains minute amounts of iron. Since the red marrow itself is an inhomogenous tissue and contains a variable amount of fat, the use of weight as a base of reference for the determination of non-hemin iron has been criticized, and references have been proposed that are more directly related to the cells of the active marrow such as deoxyribonucleic acid or protein [66]. However as far as necropsy specimens are concerned, if it is assumed that the total volume of the red marrow does not vary with the fat concentration this criticism is unjustified. Thus as regards solid marrow specimens expressed from bones obtained at necropsy it cannot be said whether weight or DNA is prefer

able as a reference base, since it is not known whether the red marrow volume, and hence the volume of the non hemin iron distribution, vary with the cellularity and fat content of the red marrow

In aspiration biopsy specimens, however other difficulties arise. Here we are not concerned with solid marrow tissue but with small fragments, usually diluted with at least 10—50 times their own volume of peripheral blood Marrow particles separated from blood may have adhering to them corpuscles weighing more than the marrow particles themselves. Weight as a reference base applied to such samples will therefore give varied results and the error may be high if the marrow specimen is small much the same will apply if protein nitrogen or phosphorus is used as a reference, since these are also present in blood and plasma. To overcome these difficulties some authors [50 65 66] have washed the marrow particles with isotonic saline or sodium citrate, removed the fluid before weighing or expressed the iron content in terms of protein [66] Such a technic, however introduces another source of error namely a partial loss of the water soluble non hemin iron fraction which usually constitutes the greater part of the storage iron in bone marrow As shown above (p 24) ferritin iron is readily extractable with saline and citrate, as well as with water Losses of the soluble non hemin iron fraction may also occur into the plasma if the marrow particles are allowed to stand for some minutes in heparinized blood even if the specimen is not washed DNA however is uneffected by admixture of blood and is therefore suitable for the purpose

Earlier attempts to determine DNA in bone marrow aspirates have been unsatisfactory because of the large amounts of tissue required for nucleic acid phosphorus determination [90] but the microbiological method [71] evolved by Løvtrup and Roos [72] is an extremely sensitive one. A method is described below for determining non hemin iron in marrow aspirates with DNA as the reference.

Method

All aparatus and utensils are carefully washed with hydrochloric acid, and thoroughly rinsed with iron free distilled water Glassware is cleaned with potassium bichromate in sulphuric acid or green soap Detergents should not be used The reagents should be of analytical grade.

Technic of aspiration

The puncture is usually performed in the corpus sterni at the level of the second inter space, or in the manubrium sterni. A 15-gauge one-inch Franzén needle with a Luer Lock fitting is used (A.B. Kifa, Solna, Sweden). After local anesthesia and infiltration of the periosteum with 2% Lidocaine the bone is pierced, usually just off the midline To obtain material for cytological and histochemical examination not more than 0.5 ml of marrow is aspirated rapidly in a 10 ml Luer Lock syringe. This syringe is quickly removed and the contents ejected on several slides. From these conventional marrow films are prepared for cytological examination. The rest of the marrow particles is fixed in neutral 10% formaldehyde for preparation of histological sections. A 20 ml Luer Lock syringe containing one drop of 5% heparin solution is immediately connected to the needle which has been left in place, and continuous suction applied the needle at the same time being gently rotated in the marrow cavity One to 3 ml of marrow and blood may be aspirated. The needle is then withdrawn and the contents ejected into

4 ml Pyrex microcentrifuge tube with glass stopper containing 0.5 ml of 3.7 % sodium citrate and 0.5 ml of dextran fraction (TDR. k205—II—B—I; Pharmacia AB). The tube is stoppered and mixed immediately

Comments. — T obtain enough material for analysis thick needle, tight system and strong suction are required. I is important to rotate the needle during suction. Aspiration from the corpus sterni at the level of the second costal interspace was found to give a better yield of marrow particles than puncture of the iliac crest (69). Large amounts of marrow were obtained from anemic subjects with bone marrow hyperplasia—often enough for duplicate determinations of non-hemin iron and ferritin iron. In cases of aplastic anemia with fatty marrow appreciable amounts of hypocellular marrow were obtained. The yield of material from healthy young non-anemic subjects with deficient iron stores sufficed for only one determination of non-hemin iron. W failed to obtain enough marrow for chemical determination from 8% of group of blood donors and 5% of group of healthy non-anemic post-gastrectomy men. In cases of myelofibrosis and in some of lymphatic leukemia the results were poor

Preparation of specimen

The marrow — blood mixture suspended in the citrate — dextran solution is mixed manually in the centrifuge tube and drawn off into a translucent semi flexible polythene tube about 30 cm long and 3 mm inner diameter Both ends of the tube are sealed by heating. The tube is divided with a razor blade into several segments of suitable length for centrifugation. The tubes are then centrifuged in a swing-out head for 10 minutes at 400 g During centrifugation the flexible tubes are fixed by insertion into rigid tubes. The centrifugation fractionates the contents of the tubes. The layers are beginning from the bottom of the tube red corpuscles with the buffy coat, the marrow of higher density a translucent fluid with a suspension of marrow particles, a column of more fatty marrow and, uppermost, neutral fat. The red-cell column is measured and the amount of plasma in the supernatant is estimated after standardizing the procedure.

The tube is cut with a razor blade just below the border of the buffy coat. The red cells are discarded. The contents of the upper part of the tube — the marrow plasma, citrate and dextran — are emptied into a 4—5 ml Potter Elvehjem homogenizer The tube is rinsed with water adjusted to pH 6.9—7 1 The material is homogenized for at least 5 minutes, and then transferred to a graded 5 or 10 ml cylinder with a glass stopper The homogenate is made up to the desired volume with water Each time an aliquot is to be drawn the homogenate is mixed carefully When the marrow content is low the homogenate is usually adjusted to 5 ml 4 ml is taken for non-hemin iron determination and 0.5 ml for the microbiological determination of DNA. Both aliquots are weighed on an analytical balance· the amounts of non-hemin iron and of DNA are calculated for 100 g of homogenate. The results are expressed in micrograms of iron per milligram of DNA. To obtain reliable results it is of prime importance to draw the aliquots quickly after careful mixing of the homogenate. The error of inhomogeneity is larger for DNA than for non-hemin iron, the former being insoluble in water

In the determination of non-hemin iron and ferritin iron by the procedures described above it is essential to correct the extinction of the color reaction for the optical density of the specimens (p. 14).

Check of the method

The DNA content was determined by the microbiological method of Lövtrup and Roos [72, 73] In this method the growth of the bacteria (*Thermobacterium acidophilum*) is determined turbidimetrically the lower limit is then 0.2 μg of DNA The average specimen of bone marrow homogenate digested for DNA determination contained about 50 μg of DNA, with a lower limit of about 10 μg The technical error of the method was calculated from duplicate determinations on 22 bone marrow homogenates obtained by aspiration The standard error for a single determination was 7% For similar determinations on 8 marrows obtained at autopsy the error was only 2 3% For these, 1—4 g of solid marrow was homogenized and the aliquots drawn for DNA digestion were large.

For 18 homogenates obtained by aspiration the DNA was determined within a week aliquots from the same homogenates were kept frozen at —23° C and used for determination of DNA after 5—7 months The average time elapsing between the first and last determination was 5.5 months Statistical analysis disclosed no significant tendency towards lower values for the older specimens. The differences between the duplicate determinations were however larger for the preserved series, for which the error was 19% against 7% for the fresh specimens.

The bone marrow homogenates prepared by the above procedure contain the buffy coat of the admixed peripheral blood and this would increase the DNA content. The magnitude of this error was determined on blood from 9 subjects. The specimens were prepared in the same way as bone marrow homogenates. The buffy coat and the supernatant plasma were separated by centrifugation in polythene tubes and transferred to graduated cylinders. The mean DNA content determined on 1 ml of blood was 20 0±2.5 μg If it is assumed that the human cell contains 8 pg of DNA [74] the corresponding figure will be 40 μg of DNA (5×10^6 white cells) In the present case a large portion of the white cells was probably sedimented with the red and discarded. Since the average sample analyzed for non hemin iron contained about 500 μg of DNA the admixture of 20 μg of DNA from the buffy coat would not alter the results. However if the marrow content is low and the admixture of peripheral blood large the results of a non hemin iron determination based on DNA may be falsely interpreted. It is therefore necessary to ensure that it is marrow that is being analyzed. The DNA content of the sample provides a good check of this In the present study only those samples were accepted that contained at least 150 μg of DNA in the homogenate after the correction for the DNA of the buffy coat if this exceeded 10% of the total in the homogenate the specimen was rejected Whenever there is only little material available a correction must be made for DNA and iron deriving from the peripheral blood

Correction for admixture of plasma iron and DNA buffy coat —The amount of peripheral blood plasma added to the

homogenate can be estimated by measuring the packed red-cell was in the polythene tubes after centrifugation. The standardization of the procedure showed that a red-cell column of 5.6 cm corresponded to 1 ml of whole blood with a hematocrit of 42—50%. From the hematocrit and the plasma iron concentration of the peripheral blood the amount of plasma iron added to the homogenate can be calculated. The amount of DNA deriving from the buffy coat is calculated from the number of millilitres of whole blood in the tubes. (The mean DNA content from one millilitre of whole blood handled in this way was 20 ± 2.5 μg.)

Example. The sum of the red-cell columns after centrifugation is 14 cm. The peripheral blood hematocrit is 50% and the plasma iron concentration 120 μg/100 ml.

Whole blood in the tubes

$$14/5.6 = 2.5 \text{ ml}$$

DNA in total homogenate derived from peripheral blood

$$2.5 \times 20 = 50 \ \mu g$$

Plasma in total homogenate

$$\frac{2.5 \times 50}{100} = 1.25 \text{ ml}$$

Iron in homogenate derived from plasma

$$1.25 \times 120/100 = 1.5 \ \mu g$$

To check the last calculation an analysis was made of the non-hemin iron of peripheral blood handled in the same way as marrow homogenate. For 9 blood specimens the mean iron content derived from 1 ml of blood was 0.8 μg ± 0.08. These values were identical with the iron content of the plasma of these blood specimens.

From the uncorrected and corrected quotients of non-hemin iron in bone marrow given in the *Appendix* it is seen that in cases with replenished iron stores the correction might be omitted, but in cases of iron depletion and appreciable admixture of peripheral blood the correction assumes importance.

Sources of error

Strict measures must be observed at all steps of the procedure to avoid contamination with iron. The microbiological determination of DNA may be unreliable if detergents are used for cleaning glassware and if the subjects have received sulphonamide or antibiotic therapy.

A large admixture of plasma with a small marrow specimen would tend to give a higher iron/DNA quotient. Hence, low marrow yields may give unreliable results. In cases of leukemia with a high white-cell count of the peripheral blood a special correction for the DNA content of the buffy coat must be applied.

In myeloproliferative disease the DNA content of the marrow sample may be high, and the iron/DNA ratio therefore too low. However, because of the increase in the total volume of the marrow in such situations, there is a dilution of the non-hemin iron even if weight is used as a reference.

If yellow marrow is analyzed too high values will be obtained with DNA as a reference. The reason for this is that in the yellow marrow there are small islands of cells, mainly reticulum

cells, which contain little iron, and as very few nucleated cells are present the iron/DNA ratio will be high. In fact, the yellow marrow contains very small amounts of iron if weight is used as a reference.

DNA versus wet weight as a reference

These experiments were carried out on 4 cadavers. About 1—4 g of red marrow was expressed from various flat bones including the sternum iliac crest, rib and vertebra. The specimens were immediately weighed and homogenized without separating the blood. The homogenate was made up to volume, and weighed homogenous aliquots were taken in duplicate or triplicate. Altogether 18 specimens were examined. The cellularity of these specimens, judged on the DNA content per 100 g wet weight, ranged from 0.267 to 0 630 g Two of the subjects had low and 2 filled iron stores.

In these specimens there was a very close correlation between the non hemin iron concentrations related to wet weight and to DNA as a reference ($r = 0\ 97$ Fig 6)

For all 4 cadavers the analysis of marrow specimens from different flat bones of the same subject disclosed an appreciable variation in the DNA concentration

These findings are in accordance with early histological studies on fat concentration in marrows from different flat bones [75] In the present study the differences in DNA concentration corresponded to variations in the non hemin iron concentration Variations were recorded whether wet weight or

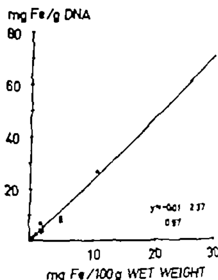
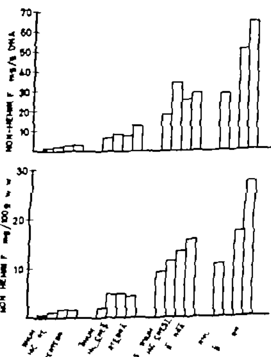


Fig. 6 Total non hemin iron in marrow (autopsy) determined on DNA related to that determined on weight as a reference



F g 7 Non hemin iron concentration in marrow of various flat bones, determined on DNA (pper) d on wet weight (lowe) a a reference. The marrow specimens were obtained from 4 cada ers.

DNA was used as the reference. The non-hemin iron concentrations in the sternal marrow were systematically lower. They were however correlated to the concentrations in the other flat bones (Fig. 7). Hence, if iron stores are to be compared in a population on the basis of determinations of non-hemin iron in the bone marrow the biopsy specimen should be taken from the same anatomic site.

Error of the method

The technical error for a single determination of non-hemin iron in marrow biopsy specimens was 4.6% (Table 15). This is slightly higher than for liver probably because of the greater difficulty in drawing homogeneous aliquots from marrow homogenates. The error of the DNA determination for the marrow aspirates was 7%. The combined error was thus 11.6%. This does not include the first steps of the procedure (preparation of the homogenate) nor is account taken of the unevenness of the distribution of non-hemin iron in the marrow.

The total error of the method, including differences in non-hemin iron concentration in different marrow specimens taken from the same bone, was examined in the following experiment. In each of 15 subjects 2 separate punctures were performed simultaneously in the same sternal segment with 2 needles. The values for the two determinations are given in Table 16. The total error for a single determination of non-hemin iron within the same region of the sternal marrow was ± 2.3 μg Fe/mg DNA, or 13.8% of the mean.

TABLE 15 *The technical error of the method of total non-hemin iron determination in bone marrow biopsy specimens calculated from duplicate determinations on the same homogenate*

Non-hemin iron (μg) in sample Range	in 100 g of homogenate Mean	n*	Standard error of single determination ± μg	Percentage of the mean
0.8 – 1.0	21.7	3	0.4	1.7
1.1 – 2.0	41.1	23	3.5	8.6
2.1 – 5.0	119.9	30	5.4	4.5
5.1 – 22.5	410.8	30	14.1	3.4
Total 0.8 – 22.5	196.9	86	9.1	4.6

*Number of bone marrow aspirates on which duplicate non-hemin iron determinations were done.

cells, which contain little iron, and as very few nucleated cells are present the iron/DNA ratio will be high. In fact, the yellow marrow contains very small amounts of iron if weight is used as a reference.

DNA versus wet weight as a reference

These experiments were carried out on 4 cadavers. About 1—4 g of red marrow was expressed from various flat bones including the sternum, iliac crest, rib and vertebra. The specimens were immediately weighed and homogenized without separating the blood. The homogenate was made up to volume, and weighed homogenous aliquots were taken in duplicate or triplicate. Altogether 18 specimens were examined. The cellularity of these specimens, judged on the DNA content per 100 g wet weight, ranged from 0.267 to 0 630 g Two of the subjects had low and 2 filled iron stores.

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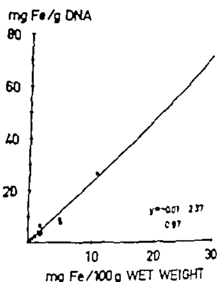


Fig. 6 Total non-hemin iron in marrow (autopsy) determined on DNA related to that determined on weight as a reference.

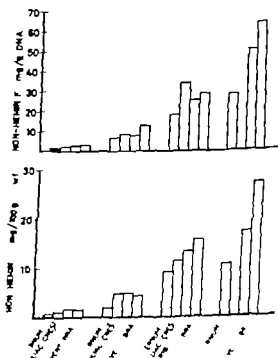


Fig. 7 Non-hemin iron concentration in marrow of various flat bones, determined on DNA (upper) and on wet weight (lower) as reference. The marrow specimens were obtained from 4 cadavers.

PART TWO

Storage Iron and Its Relation to Total Iron Binding Capacity and Serum Iron in Man

TABLE 16. *The total error of the method of non-hemin iron determination in bone marrow aspiration biopsy specimens with DNA as a reference calculated from determinations on duplicate sternal punctures in 15 subjects*[a]

Subject		Sternal puncture (μg Fe/mg DNA) I	II	d
1	E L	3 8	3.5	+ 0.3
2	A L	10 0	12.1	− 2.1
3	N B	12.9	12.5	+ 0.4
4	T E	39 8	37 7	+ [illegible]1
5	E V	18.9	19.2	− 0.3
6	N S	17.3	18.6	− 1.3
7	L R	34 0	42.0	− 8.0
8	L B	4.1	7 4	− 3.3
9	G Y	23.5	16.2	+ 7.3
10	S S	27.[illegible]	27.0	+ 0.2
11	G Y	4 4	3.4	+ 1 0
12	Å E	3 4	6.5	− 3 1
13	D B	9 6	10 4	− 0.8
14	L B	20.4	23 4	− 3.0
15	N S	14 8	16.3	− 1.5
Total		244 1	256.2	
Mean		16.3	17 1	

The standard error of a single determination was calculated according to the formula

$\sqrt{\Sigma d^2/2n}$ ± 2.3 μg Fe/mg DNA which corresponds to a coefficient of variations of 13.8%.

Introduction

The prime object of this investigation was to determine the non-hemin iron and ferritin iron in liver and bone marrow of healthy adults and in states of iron depletion and other pathologic conditions, and to examine the possibility of a relationship between, on the one hand, the non-hemin iron concentration and, on the other the total iron-binding capacity (TIBC) and serum iron.

The size of the iron stores may be greatly affected by exogenous factors that can result in either a positive or negative iron balance, for instance loss of iron through bleeding, a deficiency or excess of iron in the diet, blood transfusions and parenteral administration of iron. Endogenous factors that may influence the iron stores are anemia that is not due to a negative iron balance and that can augment the iron stores, and an increase in the blood volume in the growing body and polycythemia, which can diminish the stores. If all these factors were eliminated the remaining variations in the iron stores would be due to the individual variations in external iron balance i.e. in iron absorption from the gut, and physiological iron losses. Thus, to obtain a true picture of the physiological variation in the iron stores all the above factors must be taken into account. The same is true of comparison of pathological cases with controls.

All previous studies of non-hemin iron and/or ferritin iron in liver have been carried out on autopsy specimens and have been concerned chiefly with pathological cases, usually of malignant, infectious and toxic diseases, and without due regard for factors governing the iron stores [76—80] The series in a few investigations have contained cases of sudden death in accidents, subjects that may be regarded as "normals" [78—80] It was considered of interest to compare these figures with those of the present series of biopsy specimens from subjects whose history blood values and other factors of importance for the iron metabolism were known.

The iron stores are built up during childhood and adolescence through a positive iron balance, in this way the iron depots are gradually enlarged until equilibrium is established. It is believed that the total body iron in the normal adult male is maintained within fairly constant limits. Since there is no known excretory regulation of iron [81 82] this implies a control of iron absorption so as to maintain the physiological level in the body Absorption studies have shown that there is a wide variation in the rate of absorption of iron in apparently healthy subjects [83—85] The results of clinical and experimental work suggest that absorption may be in some

Other groups Various groups of disturbed iron metabolism and groups of infectious or toxic disease. These groups are subdivided with respect to anemia, sex, menstruation, recent or past hemorrhage and exogenous iron load.

The group is given in the tables (*Appendix*) containing the relevant data for each subject. A more detailed description of the material is given in the appropriate section.

Anemia is defined in the present investigation as a hemoglobin level below 13.5 g/100 ml for men and below 11.5 g/100 ml for women determined in the morning on venous blood without stasis [93]

Iron deficiency anemia is defined as anemia in which the only factor limiting the production of hemoglobin is a lack of available iron. A clinical diagnosis of iron deficiency anemia was made if anemia persisted in spite of the absence of hemorrhage or signs of infectious disease, and there was a rise in the hemoglobin concentration of at least g 100 ml after 3 weeks of efficient iron treatment [94 95]

The term iron deficiency anemia is also appropriate when the total body iron is insufficient to provide normal mass of hemoglobin [96] This definition, although from the stand point of iron metabolism more general than the former one, is however hardly applicable in clinical work. According to this definition the term iron deficiency anemia will include cases whose actual cause of retarded hemoglobin formation is some other disease but whose stores are insufficient for complete restoration of the hemoglobin mass

Post-hemorrhagic anemia includes some cases of true iron deficiency anemia in which this diagnosis could not be established in accordance with the above criteria.

History of hemorrhage Hemorrhage at any time before the study

Recent hemorrhage Hemorrhage less than 3 months before the study

Old hemorrhage Hemorrhage at least one year before the study

Past hemorrhage Hemorrhage at least 3 months before the study

Anemia of infection and an infectious or toxic state without anemia. The basic diseases of these subjects were acute infections with fever systemic diseases, chronic liver and kidney diseases and malignant tumours.

Methods

The blood specimens were taken from fasting subjects in the morning, for the patients from the Surgical Department usually on the day before operation. The specimen was obtained by venipuncture of the antecubital vein with a polished stainless steel needle. The puncture was made under slight stasis, which was released immediately after the needle had been inserted. A sample of 25 ml was taken for determination of serum iron and total iron binding capacity followed by one of about 10 ml in a heparinized tube for determination of hemoglobin. The latter sample was mixed, placed immediately on a mechanical blood-mixing apparatus and left there until aliquots could be drawn for hemoglobinometry

Determination of hemoglobin

Hemoglobin was determined as cyanmethemoglobin. Two portions of 20 µl

measure regulated by the size of the iron stores [86 87] but how this might occur is not known Laurell [88] has proposed the relative saturation of iron binding protein as one possible mechanism in the regulation of iron absorption, and this view has recently found support in experimental studies on man and laboratory animals [89 90] In other animal experiments, however conflicting results have been reported [91 92] An examination was therefore made of the relationship between, on the one hand storage iron concentrations in the liver and bone marrow and on the other the transferrin and serum iron levels.

In recent years some authors focused interest on the proportion of ferritin iron to total non hemin and hemosiderin iron The normal ranges are not known however The few studies reported are concerned with autopsy specimens, and it is possible that changes in ferritin content may occur during the agonal shock and post mortem

The next problem to be investigated was the relation of histochemically stainable iron in the form of reticular hemosiderin and sideroblasts, to the chemically determined non-hemin iron in bone marrow the histochemical method is at present the only practical one for assessing iron stores.

The material for this investigation was derived from three sources (*a*) Patients admitted to either of the two Surgical Departments of this Hospital for an upper abdominal operation (*b*) Patients admitted to the First Medical Department of this hospital for a variety of disorders (*c*) Blood donors and male out patients who had previously undergone subtotal partial gastrectomy by the Polya technic.

Grouping of subjects and definitions

Factors that can, or are believed to be able to influence the size, composition and distribution of iron stores and level of iron binding capacity and serum iron are of greater importance in the present context than the clinical diagnosis of the disease. Some conditions influence only one of these parameters for instance, blood loss may directly influence the size of the iron stores even if it occurred many years previously It is improbable, however that an old hemorrhage can directly influence the level of TIBC or serum iron or the proportion of ferritin or hemosiderin except as an indirect effect of a reduction in the iron stores themselves. On the other hand acute infection of several days duration without anemia would hardly alter the quantity of storage iron but may change the serum iron and TIBC levels or alter the proportion of ferritin to hemosiderin

The following main grouping was recognized in the study

Controls Hematologically normal persons with no history of hemorrhage but possibly with mild infection of short duration such as *cholecystitis*, but no other complication

Basal subjects Hematologically normal persons who had not had hemorrhage within the last year and had no signs of infection or toxicity at the time of study

Other groups Various groups of disturbed iron metabolism and groups of infectious or toxic disease. These groups are subdivided with respect to anemia, sex, menstruation, recent or past hemorrhage and exogenous iron load.

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phate (Mohr's salt) was used as a standard. For each serum iron analysis, two duplicate standard dilutions of 100 and 20 µg Fe/100 ml were run. The variations of the extinctions of the reagent blanks and of the iron standards on different days of analysis was negligible. The serum was kept at 4 C and analyzed not more than 4 days after sampling. Frozen serum was not used. All determinations were performed in duplicate.

The error of a single determination calculated from 60 random duplicate determinations was ± 1 8 µg/100 ml, which corresponds to a coefficient of variation of 1.3 /

Determination of total iron binding capacity of serum

The total iron binding capacity was determined by the method of Peters *et al* [99]

Resin As the small beads present in Amberlite Ira 410 anion resin exchanger may interfere with efficient separation of the resin from the supernatant fluid, they were discarded by differential sedimentation during the washing of the resin with water

The ferric ammonium citrate solution was kept in the refrigerator at 4 C and checked fo its iron content. Fresh solutions were made every month, or more often if flocculation or growth of bacteria was observed. The iron concentration of the solution was 0.050 mg/ml and throughout the study the amount added to saturate 1 ml of serum was 5 g No correction was made in the final calculation for the unbound iron not removed by the resin, which according to Peters *et al* amounts to 4 per cent of the difference between the ferric ammonium citrate added and the unsaturated iron binding capacity of serum (UIBC) If greater amounts of iron are added to serum this error may be appreciable even though much larger amounts of iron are removed efficiently by the resin when no serum is added.

The sample From the fresh and clear serum 2 or 3 aliquots of 1 ml were transferred to tubes which were wrapped with a double adhesive polyethylene film and kept at —23 C until analysis — usually within 2 weeks of sampling.

Test serum. One millitre volumes of a batch of fresh serum obtained from a blood donor were preserved in tubes at —23 C. Whenever a TIBC determination was performed two or three of these tubes were taken for analysis as a check of the procedure. Such a serum batch was usually used for 3 to 6 months.

Reagent blank Three reagent blanks prepared in the same way as the serum sample but containing water instead of serum were run with each analysis. At the same time 3 reagent blanks used for the determination of serum iron were run. When analyzed for iron the difference in the extinctions of these two pairs of blanks (one containing ferric ammonium citrate and treated with the resin, the other not) was negligible This served as check of the efficiency with which the resin removed iron. The extinctions of the serum samples were corrected for that of the resin-treated blank.

Determination of iron. The determination of the serum iron after saturation,

of blood were added to 10 ml of Drabkin's solution [97] (dilution 1:251) and after at least 20 minutes the extinction was read against the reagent solution at a wave length of 545 mμ in a Beckman *Model B* spectrophotometer. By multiplying the extinction by a factor of 35.7 the concentration of hemoglobin in grams per 100 ml is obtained. All hemoglobin determinations were performed in duplicate on the same sample of blood. The error of a single determination calculated from 50 random duplicate determinations was ± 0.14 g/100 ml which corresponds to a coefficient of variation of 1.0%.

Determination of serum iron

The serum iron was determined by an unpublished method of Laurell [98].

Reagents

3 *N* hydrochloric acid

Trichloroacetic acid 20% (redistilled)

One per cent *o*-phenanthroline solution in absolute alcohol

Thioglycollic acid 89%

Sodium acetate — ammonia mixture

600 g of sodium acetate is dissolved by heating in water and the volume is made up to 1000 ml with water. To the solution 20 ml of glacial acetic acid and 40 mg of *o*-phenanthroline are added. When the *o*-phenanthroline has dissolved 200—300 mg of sodium hyposulfite is added. The solution is allowed to stand for 24 hours. About 400 mg of activated charcoal is added and the solution shaken. After an hour it is filtered through double filter paper to an iron-free bottle. No charcoal particles should be present in the solution. To 1000 ml of the iron free sodium acetate solution about 75 ml of 25 % ammonia is added. The amount required is adjusted by checking the pH of the resulting solutions. From a mixture of 2 ml volumes of 3 *N* hydrochloric acid, 20% trichloroacetic acid and water 4 ml is drawn and added to 2 ml of the sodium acetate—ammonia mixture. The latter solution corresponds to the reagent blank before the addition of the color reagent and the reducing agent employed in the procedure described below. The pH of this solution should be 4.8—5.0. Ammonia is added to the sodium acetate solution until the required pH is obtained.

Procedure. Two millilitres of 3 *N* hydrochloric acid is transferred to a 15 ml tube to which 2 ml of serum is added *slowly so as to ensure a homogenous* precipitation of protein. The tube is shaken gently and allowed to stand for 10 minutes. 2 ml of trichloroacetic acid is added, the mixture stirred with a glass rod and allowed to stand 10 minutes. It is centrifuged for 20 minutes at 3000 r.p.m. From the clear supernatant 4 ml is drawn carefully and transferred to a tube containing 2 ml of sodium acetate—ammonia mixture. To the last 0.1 ml *o*-phenanthroline is added, the tubes are shaked vigorously and 0.2 ml of thioglycollic acid is added. The solution is stirred with a glass rod and allowed to stand for at least one hour by which time the color will be fully developed. The extinctions are read off at 510 mμ against water in a 50 mm cell in a Beckman Model B spectrophotometer or, as was usually done, in an Eppendorph spectrophotometer with filter no. 492, photocell 90 B and a mercury lamp. To correct for the iron content in water and the reagents, 3 reagent blanks were run with every serum iron analysis, and their mean optical density was substracted from the serum samples. No correction was made for the serum blank. The iron content of the serum was read from a calibration graph drawn for appropriate dilutions of a standard stock iron solution handled identically to the serum sample. Ferrous ammonium sul

In some cases a non-linear model was found to be the more suitable for estimating the relation between two variables x and y and then a semiparabolic function fitted the observations better than an exponential regression function. There was a negative relationship between the values x and y. The function $y = \alpha + \beta x + \gamma x^2$ was fitted to the observations and this gave an estimate $\bar{y} = a + bx + cx^2$. This function was used as a model for the observations up to the value of x giving the minimum for the function. For higher values of x it was considered that y was constant and equal to y (min). This value is calculated as follows. The function has a minimum for $x = -b/2c$. This value is inserted in the function to give

$$y\ (\text{min}) = a - b^2/4c$$

To obtain a measure of the goodness of fit in these cases $r^2 = \frac{s_y^2 - s_d^2}{s_y^2}$ was calculated, where the sign of r is determined by the slope of the curve. In principle this r is analogous to that in the linear regression: this is the reason for not introducing different symbols. The condition for accepting the model was that r should be significant at the 5% level.

The majority of statistical calculations are concerned with the problem "Are these groups equal to that group?" (with respect to the association between x and y). The technic used here is as follows. For any individual a value y_{calc} was calculated by inserting the value of x for that individual in the regression function calculated from the basal group. The difference $d = y_{calc} - y_{obs}$ (y_{obs} is the observed value) was analyzed by the t test.

Other statistical operations, such as analysis of differences between means, correlation coefficients, differences between correlation coefficients and regression coefficients, comparison of variances and calculation of method errors have been carried out by the standard procedures [100].

and treatment with the resin and buffer solution was performed by the above method of Laurell Four millilitres of the supernatant was transferred to a tube to which 1 ml of 6 *N* hydrochloric acid and 1 ml of 40% trichloroacetic acid were added (instead of the 3 *N* and 20% respectively) and the procedure continued as described above under *Determination of serum iron* The TIBC value was obtained by correction of the serum iron value read from the calibration graph for the dilutions of serum used

The method has been in use for 3 years and given satisfactory and accurate results consistent with the clinical diagnosis. The technical error of the method estimated from 100 random duplicate determinations was ± 5 1 μg/100 ml which corresponds to a variation coefficient of ± 1 6% The error of the mean of a duplicate determination was 1 1% All TIBC values given in the present investigation are means of 2 determinations.

The standard error of the mean of a duplicate determination calculated from determinations on frozen test serum batches which were analyzed at different times and over periods of 4 to 7 months varied from ± 1.2 to ± 12 7 μg/100 ml or a coefficient of variation of ±0 3—3.3%.

The sternal puncture was usually made in the morning at the time of blood sampling The bone marrow aspiration was always performed from the second upper sternal interspace The liver biopsy specimen was taken by the surgeon from the margin of the right lobe of the liver at the start of the operation, before any blood was lost The specimens usually weighed 0.5—1 0 g wet weight. The peripheral blood was dried from the surface of the tissue, the connective tissue adhering to the capsule separated and the specimen put into a stoppered 4 ml bottle. It was weighed immediately and stored at —23°C. It was observed that even if left in a closed container for only some hours smaller specimens sometimes diminished in wet weight by more than 25% The wet weights of these specimens were obtained from the dry weights according to a formula derived from a correlation curve drawn from 35 determinations of wet and dry weights.

In the account of the studies and the statistical analysis the total non hemin iron concentration is given on the basis of the dry weight, and in the tables of the *Appendix* on the dry and wet weights

Statistical methods

The following notation is used.

n number of observations or pairs of observations

$\bar{x}$ $\bar{y}$ means

s^2 s_y^2 variances

s s_y standard deviations

$s_{\bar{x}}$ $s_{\bar{y}}$ standard errors of means

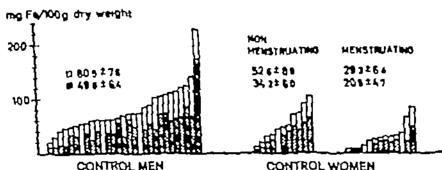
s_y^2 residual variance

s_y residual standard deviation

$\bar{y} = a + bx$ estimate of the linear regression of y upon x

r the correlation coefficient between two linearly related variables.

NON HEMIN IRON IN SURGICAL LIVER BIOPSIES



NON HEMIN IRON IN BONE MARROW BIOPSIES

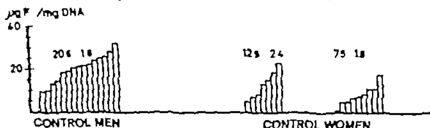


Fig 8 Total non-hemin and ferritin iron concentrations in li er and bone marrow biopsy specimens f the control subjects. The whole bars indicate the total non-hemin iron, and the hatched areas the ferritin iron. The values are means with its standard errors.

cantly higher for the male group than fo the group of non-menstruating women. The values for the latter group were also significantly higher than those for the menstruating group (Fig 8 Table 17)

TABLE 17 *Material for study of the total non-hemin iron and ferritin iron concentrations in liver biopsy specimens f the ontrol subjects. The d fferences between the means f the 3 groups were examined by the t-test. The ranges are given in parentheses.*

on-hemin iron in liver	n	Control men $\bar{x}$ (mg dry t)	n	Control women non-menstruating $\bar{x}_2$ (mg % dry t)	Test of means $\lvert t \rvert$	P ( )	n	Control women menstruating $\bar{x}$ (mg dry wt)	Test of n $\lvert t \rvert$	
tal non-hemin n	29	80.5 7.6 (19.0 227.0)	11	52.6 _ 8.9 (11.9 106.6)	3.03	< 0.05	13	29.3 ± 6.4 (4.0–83.2)	2.13	<
rritin iron	26	49.8 6.4 (10.8 174.4)	10	34 ± 6.0 (11.1–64.5)	1.786	0.10 > P > 0.05	10	20.5 ± 4.7 (4.5–58.4)	1.80	0.10
rcentage of rritin iron	26	58.8 ± 2.8 (32–88)	10	63.5 ± 9.9 (46–79)	0.4	> 0.10	10	70.2 ± 5.6 (33–100)	0.5	>

Total Non-Hemin and Ferritin Iron in the Control Subjects

Liver

None of the subjects in this group had a history of abnormal blood loss. They were all in good health and in a good nutritional state There was no abuse of alcohol or salicylates. The size of the menstrual blood loss was judged to be normal Cases of menorrhagia, metrorrhagia myoma or prolapse of the uterus were excluded The clinical diagnosis for these patients was

(1) Cholelithiasis with no history of cholecystitis. The walls of the gall bladder were thin[1]

(2) Cholecystopathy (*i*) a history of acute cholecystitis of short duration and no symptoms of infection in the 5 weeks before examination (stages III—IV) (*ii*) acute or subacute cholecystitis at the time of operation (stages 1 and 11) *There was a thickening of the gall* bladder wall[2]

None of these patients had jaundice at the time of examination nor were any histologic alterations in liver structure noted. These patients were regarded as controls so far as the quantity of storage iron is concerned but they were not regarded as basal for analysis of the relationship between non-hemin iron and serum iron-binding capacity or serum iron nor for the study of the relation between the two non-hemin iron compounds.

(3) Uncomplicated gastric ulcer in otherwise healthy subjects. Cases with stenosis, vomiting anorexia or penetrating or callous ulcers were excluded.

The whole control group comprised 53 subjects — 29 men, 11 post-menopausal and 13 menstruating women. The ages of the males ranged from 24 to 77 years, mean 46 The clinical diagnosis was uncomplicated gastric ulcer in 14 cases, uncomplicated cholelithiasis in 7 and chronic cholecystopathy in 8 The age range for the post menopausal women was 46—77 years, mean 60 The diagnosis was uncomplicated cholelithiasis in 6 cases, chronic cholecystopathy in 4 and uncomplicated gastric ulcer in one.

The menstruating women were aged 17—46 years, mean 33 There was uncomplicated cholelithiasis in 8 cases, chronic cholecystopathy in 3 and uncomplicated gastric ulcer in one

Results

The mean total non-hemin iron concentration in liver tissue was signifi

[1] *Cholecystitis chronica primaria* in the classification by Edlund and Zettergren [101]

[2] Cholecystitis chronica secundaria stages I—IV [101]

groups. Within the male group the gastric ulcer sub-group (14 cases) did not differ from the other 2 sub-groups. Hence these 3 diagnostic sub-groups could be treated as a single group

Bone marrow aspirates

The group examined comprised 31 cases — 15 men and 7 post-menopausal and 9 menstruating women.

The men ranged in age from 23 to 62 years, mean 46. The clinical diagnosis was uncomplicated gastric ulcer in 6 cases, uncomplicated cholelithiasis in 7 and coronary artery disease and thyreotoxicosis in remission in one case each.

For the menopausal women the age range was 45—78 years, mean 59 years. There were 6 cases of uncomplicated cholelithiasis and one of gastric ulcer The menstruating women were 23—50 years of age, mean 33 Four had uncomplicated cholelithiasis, 2 gastric ulcer and 3 were healthy volunteers.

TABLE 19 *Material for study of the total non-hemin iron in marrow aspirates of the control subjects. Test of the means of the 3 groups. The ranges are given in parentheses.*

	Control men		Control women non-menstruating		Test of means		Control women menstruating		Test of m…	
		$\bar{x}$		$\bar{x}$	$\frac{\lvert t \rvert}{-}$	$P_{(t)}$		$\bar{x}_s$	$\frac{\lvert t \rvert}{t_s}$	P
…l non hemin iron …ne marrow (Γ mg DNA)	15	20 6 1 8 (9 4 36.9)	7	12.5 ± 2.4 (4 4 17 4)	2.7	< 0.01	9	7.5 ± 1.6 (0—17 1)	1.7	>

Results

The concentrations of non-hemin iron for these subjects are shown in Figure 8 and the means, the ranges and statistical significance are given in Table 19 The mean was significantly higher for the men than that for the post-menopausal women, but the mean for the latter was not significantly higher than that for the menstruating group.

Non-hemin iron in relation to age

LIVER. — For the 29 men there was no correlation between the concentration of total non-hemin iron in liver tissue and their age ($r = 0\ 18$ $t = 0\ 99$) When the group of males were divided into the 2 classes 24—41 and 42—77 years of age with 14 and 15 subjects, respectively the means were not significantly different (70 9 ± 6.7 and 89.5 ± 13.2, resp.). The slightly higher value for the older group was due to the inclusion of one extremely high value (no 16) Nor was there any correlation with age for the combined group of women, in spite of the significant difference between the means for the menstruating and post menopausal groups.

The mean ferritin iron concentration was significantly higher for men than for the whole group of women and almost significantly higher than for the non-menstruating women. The mean ferritin iron for the latter group was almost significantly higher than that for the menstruating women

An approximate calculation of the absolute amount of total non hemin iron in liver gives means of 400 mg for men 235 for menopausal women and 130 for menstruating women (on the basis of liver weights of 1700 g for men and 1500 for women and 30% dry weight)

For the combined control group the ferritin iron was 64.7% of the total non hemin iron

The percentage of ferritin in total non-hemin iron was not significantly different for the male group and non menstruating women nor for the non menstruating and menstruating groups (Table 17) The difference between the male group and the menstruating women was not statistically significant.

Since it is possible that cases of gastric ulcer even with no history of hemorrhage might loose occult blood, and that cases of acute or chronic cholecystitis might differ from those with no evidence of infection a statistical analysis was performed of these clinical sub-entities within each of the 3 control groups (Table 18) In none of the 3 control groups was there any difference between the 3 diagnostic sub-groups as regards the mean total non hemin iron or the mean ferritin iron. The post-menopausal and the menstruating group each contained one case of uncomplicated gastric ulcer (nos.[1] 35 and 48 resp) the values for which fell within the ranges of the normal distribution for the respective

[1] See tables in *Appendix*

TABLE 18 *Analysis of the means of the three diagnostic subentities within each of the control groups*

min iron	n	Uncomplicated gastric ulcer $\bar{x}_1$ (mg % dry wt)	n	Uncomplicated cholelithiasis $\bar{x}_2$ (mg % dry wt)	Test of means $\frac{\|t\|}{\bar{x}_1-\bar{x}_2}$	$P_{(t)}$	n	Chronic cholecystitis $\bar{x}_3$ (mg % dry wt)	Test of means $\frac{\|t\|}{\bar{x}_2-\bar{x}_3}$	$P_{(t)}$
...										
hemin iron	14	79 0 ± 9 0	9	86.6 ± 20.5	< 1	> 0.10	6	75 0 ± 7 1	< 1	> 0.10
on	13	40 6 ± 5.6	8	65.2 ± 17 8	< 1	> 0 10	5	49 4 ± 6.5	< 1	> 0.10
...										
...										
hemin iron	1	19 7	6	55.6 ± 22.4			4	56.4 ± 14.3	< 1	> 0 10
on	1	11 1	5	36.6 ± 9 4			4	37 0 ± 8.4	< 1	> 0.10
...										
...										
hemin iron	1	65.9	8	20.7 ± 4 2			4	37 4 ± 16.1	1 0	> 0.10
on	1	21 6	5	15.9 ± 3.3			4	26.0 ± 11 4	< 1	> 0 10

groups. Within the male group the gastric ulcer sub-group (14 cases) did not differ from the other 2 sub-groups. Hence these 3 diagnostic sub-groups could be treated as a single group.

Bone marrow aspirates

The group examined comprised 31 cases — 15 men and 7 post-menopausal and 9 menstruating women.

The men ranged in age from 23 to 62 years, mean 46. The clinical diagnosis was uncomplicated gastric ulcer in 6 cases, uncomplicated cholelithiasis in 7 and coronary artery disease and thyreotoxicosis in remission in one case each.

For the menopausal women the age range was 45—78 years, mean 59 years. There were 6 cases of uncomplicated cholelithiasis and one of gastric ulcer. The menstruating women were 23—50 years of age, mean 33. Four had uncomplicated cholelithiasis, 2 gastric ulcer and 3 were healthy volunteers.

TABLE 19. *Material for study of the total non-hemin iron in marrow aspirates of the control subjects. Test of the means of the 3 groups. The ranges are given in parentheses.*

		Control men		Control women non-menstruating	Test of means			Control women menstruating	Test of m[…]	
		$\bar{x}$		$\bar{x}$	$\frac{\lvert t \rvert}{\ldots}$	$P_{(t)}$		$\bar{x}_3$	$\frac{\lvert t \rvert}{\ldots}$	
[..]al non hemin iron [..]one marrow ([..] Γ mg DNA)	15	20.6 ± 1.8 (9.4—36.9)	7	12.5 ± 2.4 (4.4—17.4)	2.7	< 0.01	9	7.5 ± 1.6 (0—17.1)	1.7	>

Results

The concentrations of non-hemin iron for these subjects are shown in Figure 8 and the means, the ranges and statistical significance are given in Table 19. The mean was significantly higher for the men than that for the post-menopausal women, but the mean for the latter was not significantly higher than that for the menstruating group.

Non-hemin iron in relation to age

LIVER. — For the 29 men there was no correlation between the concentration of total non-hemin iron in liver tissue and their age (= 0.18, t = 0.99). When the group of males were divided into the 2 classes 24—41 and 42—77 years of age with 14 and 15 subjects, respectively, the means were not significantly different (70.9 ± 6.7 and 89.5 ± 13.2, resp.) The slightly higher value for the older group was due to the inclusion of one extremely high value (no. 16). Nor was there any correlation with age for the combined group of women, in spite of the significant difference between the means for the menstruating and post-menopausal groups.

Nor as regards ferritin iron was there any correlation with age.

There was no significant correlation between age and the percentage of ferritin iron in the total non hemin iron of liver tissue.

Bone marrow — For the 15 control men ther was a significant correlation between total non hemin iron in the marrow and age (Fig 9) As the figure shows, the regression was due mainly to a rise in non hemin iron at the lower ages. After the age of 55 there was no further tendency for the non hemin iron concentration to increase.

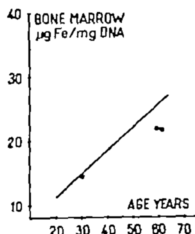


Fig 9 Correlation between non-hemin iron concentration in marrow biopsy specimens and age of the control men. $\bar{y} = 4.18 + 0.36x$ = 0.68 $t = 3.3$ $P < 0.01$ $n = 15$

Discussion

The only reported normal values for total non-hemin iron concentration in liver tissue relate to persons dying in accidents. It is assumed by the authors that the iron balance had been normal Some of these series were too small for any meaningful comparison

In Schairer and Rechenberger's [78] series of 18 males killed in accidents at ages ranging from 14 to 82 years the mean concentration was 95 ± 12.7 mg/100 g (converted to dry weight, and assuming a water content of 70%) This value is not significantly different from that of 80.5 ± 7 6 mg for the present study Eight of the subjects were young males (aged 14—22 years) and their values were significantly lower than for the others. The mean for the remaining 10 cases 124 7 ± 16 9 mg was significantly higher than the present mean the difference is probably due to dissimilarities in the analytical methods. Schairer and Rechenberger determined total iron on exsanguinated livers by wet ashing It is hardly possible to free the liver from blood by perfusion with saline, especially in autopsy specimens that are undergoing decomposition [50 102]

In a more recent investigation by Morgan and Walters [79] both total non-hemin iron and ferritin iron were determined in liver specimens from 21 subjects (18 men 3 women) killed in accidents. The means given by these authors converted to dry weight, are 81 5 ± 10 7 mg of total non-hemin iron/100 g and 54 4 mg of ferritin iron The percentage of the ferritin iron fraction was 66 8 These values are almost identical with those of the present study The chemical methods of the 2 investigations are comparable Morgan and Walters means included values for 3 women, but it is unlikely that these affected the mean for the whole group

The similarity in the results is of special interest since the two studies

were carried out in different parts of the world (Australia and Sweden) and the occurrence of geographic differences has been suggested [103]

Sex differences in the non-hemin iron content of liver tissue were studied for the first time by Schairer and Rechenberger [78] In an anlysis based on 193 consecutive autopsies without regard to history of blood loss or clinical diagnosis these authors found significantly higher non-hemin iron levels in the liver for men than women. In spite of invariably lower mean concentrations in women of all 6 age classes, the differences were not statistically significant. The greatest differences occurred in the 20—40 years group and this the authors ascribe to the high iron requirements at this period of reproductive life The absence of significance may be due to the heterogeneity of the material. Roth, Jasinski and Bidder [104] also observed a tendency towards lower values in women. In the present material a significant difference was found not only between the sexes but also between men and post-menopausal women and between post-menopausal women and menstruating women. The persistent difference between men and non-menstruating women whose mean age was 10 years after the menopause suggests that this time is not enough for the stores to build up The difference may also be due to other factors, one of which may be a relatively less efficient absorption from the gut at higher than at lower ages. The results of the study on bone marrow are in close agreement with those for liver tissue (Table 19) Accordingly even in the bone marrow there was still a significant difference between the men and the post menopausal women with an average age of 59 years.

There is general agreement that the liver iron increases during the first month of life, and falls to a minimum during the second year after which there is a gradual increase which continues during adolescence [80, 105 106] However no study has been made of the relationship between the size of the iron stores and age during adult life in the normal subject. It is of biological interest to know whether the iron stores in the adult remain fairly constant, or whether there is a slow accretion of non-hemin iron during the rest of life. Such a study must, of course, be carried out on normal males with a normal iron balance in the past.

In a study of the life curve of non-hemin iron in the kidney Brückman and Zondek have shown that the level was fairly constant from the third decade up to old age [80] The series was however small and heterogeneous, comprising both sexes. Besides, the kidney is not the main storage organ for iron and may therefore not well reflect the iron stores. In an analysis of the values for non-hemin iron concentrations over the age range of 10—80 years Schairer and Rechenberger found in both sexes a significant rise with age in the liver but not in the spleen [8] The analysis was performed on an unselected autopsy material which included *many* cases of severe infection, sepsis and malignant tumours. The hemoglobin levels and the amount of blood transfused before death

were not reported It may be inferred that the older the patient the more severe the debilitating disease and the degree of anemia of infection and this may account for the increase in iron stores with age However the values obtained by the above authors for the 18 males who died in accidents give a significant correlation with age, but if the 8 adolescents are excluded the correlation will not be significant.

The linear regression calculated for the 18 cases of the above authors is $\bar{y} = 6.5 + 0.59x$ $r = 0.73$; $P < 0.001$ For the 10 subjects above 22 years of age the correlation coefficient of 0.50 is not significant.

Meier *et al* studied the age relations of total iron (non-hemin and hemoglobin iron) and ferritin iron in livers from male subjects who died in accidents or committed suicide [102] The series included representative numbers of elderly subjects between 60 and 90 years old. For ferritin there was a significant rise up to the age of 59 years, with significant differences only between the 10—19 and 20—29 year classes. After the sixth decade there was a gradual decrease in the means, but this was not significant

In the present investigation no significant correlation with age was found for total non hemin iron or ferritin iron concentration in liver tissue. The low coefficient of correlation ($r = 0.18$) for the group of 29 adult males suggests that the results would not be essentially different for a larger series. This seems to be in accordance with the findings of Morgan and Walters, who mentioned that the "normal subjects" aged 20—50 had higher values than either the younger or older groups. There was however a significant correlation between the non hemin iron concentration in the bone marrow and age for the male subjects (Fig 9) The numbers of observations are too small for conclusions to be drawn but here too there was no tendency for a further rise after the age of 55 years.

These results support the belief that the iron stores in the adult remain within fairly constant limits. This would imply the existence of a mechanism that regulates the iron balance. It is beyond the scope of this study to discuss the factors that may be involved here but it is feasible that at least one of them is the iron stores themselves [86 87 103] The observations of Meier and Morgan & Walters [79 102] that the iron concentration in the liver tends to decrease in the old subjects is striking in view of the decrease in the muscle and liver masses in advanced age [107] This diminution of iron in the livers of the elderly can be ascribed to a negative iron balance and/or a redistribution of iron stores. A less effective absorption of food iron together with a reduced intake has been suggested above as a possible reason for differences in non hemin iron concentrations between men and post menopausal women Another possible factor may be loss of iron as a result of the greater fragility of the capillaries at advanced age.

It has been suggested that the percentage of ferritin decreases with advancing age [108] but neither the present study nor those of Morgan & Walters and Meier provide support for this view

Total Non-Hemin and Ferritin Iron in Iron Depletion

Liver

This study was performed on a group of subjects with a history of abnormal blood loss and a group of patients who had previously undergone partial gastrectomy

Most of the patients with a history of hemorrhage had been admitted to hospital to undergo operation for gastric ulcer The subjects were divided according to whether the hemorrhage was recent or had occurred at least one year before the examination Unless otherwise stated, none of them had anemia at the time of the examination.

For 6 male subjects there was a probable diagnosis of iron deficiency anemia on the basis of the history and laboratory data. The anemia was slight — mean hemoglobin 12.1 g/100 ml.

The group that had undergone partial gastrectomy (8 post Polya, 4 post B llroth I) all men had been admitted for gall-bladder disease. For all but one (no 111) of the Polya group the time elapsing after the gastrectomy exceeded 5 years. The mean hemoglobin value was 13.2 g/100 ml. Two patients (nos. 112 113) had mild anemia of infection, and two (nos. 107 108) slightly low hemoglobin values, which did not rise significantly after administration of parenteral iron. Three of the Billroth I patients had undergone gastrectomy more than 13 years and one 2 years before the examination. Their mean hemoglobin value was 14 1 g/100 ml.

Result

Iron deficiency anemia.—The mean values of total non-hemin and ferritin iron in "iron deficiency anemia" were 5.5 mg and 2.0 mg per 100 g of dry liver tissue. These means are significantly lower than those of all 3 control groups. There was no overlapping between the individual subjects of the iron deficiency anemia group and those of the groups of control men and non-menstruating women. There was, however overlapping with the control menstruating subjects with regard to the total non-hemin iron but not to the ferritin iron.

History of hemorrhage. — There was no significant difference between the means for the males with recent hemorrhage and those who had had hemorrhage in the past. The appreciably higher mean in the latter group is due to one extremely high value (no 89) and if this is excluded the means for both groups are nearly the same. It is noteworthy however that the percentage

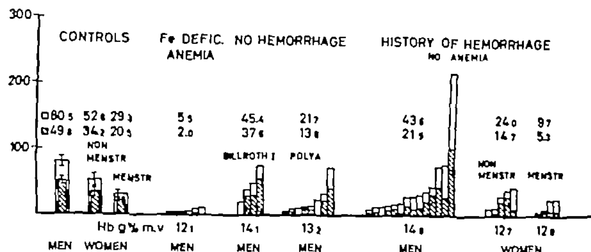


Fig 10. Total non-hemin and ferritin iron concentrations in liver biopsy specimens in states of iron depletion. The whole bars indicate the total non-hemin iron and the hatched areas the ferritin iron. The values are means of the group. The group of males with a history of hemorrhage comprises both recent and old hemorrhage of table 20.

of ferritin in total non hemin iron for the group with *recent* hemorrhage was significantly lower than for the controls ($t = 2.47$ $P < 0.01$). For the two hemorrhage groups combined the respective means of the non hemin and ferritin iron were significantly lower than for the control group ($t = 2.2$ $P < 0.05$ and $t = 2.82$ $P < 0.01$ respectively).

For the small groups of women with abnormal iron losses the means for the total non hemin and ferritin iron were significantly lower than for the respective control groups. All the non-menstruating women had had hemorrhage at least one year previously, of the menstruating women 2 had had recent hemorrhages; the percentage of ferritin was lower for the latter group.

Partial gastrectomy. — The post Polya gastrectomy group had significantly lower total non hemin and ferritin iron concentrations than the control group. For the post Billroth I group there was a significant difference from the control group as regards the total non hemin iron but not for ferritin iron. The percentage of ferritin in the total non hemin iron for the gastrectomy cases was not significantly different from that for the controls.

Comparison of the Polya and Billroth I groups disclosed that the means of the former were only almost significantly lower for both total non hemin iron and ferritin iron, probably because of the small number of cases.

...LE 20. Material for study of total non-heme iron and ferritin iron concentration in liver biopsy specimens in states of iron depletion.

Group	Sex	Hemoglobin (g/100 ml)	n	Total non-heme iron in liver T (mg/g dry wt)		Test of means[a] |t|	P	n	Ferritin iron in liver F (mg/g dry wt)		Test of means[a] |t|	P	Percentage ferritin iron in total non-heme iron
...ntrol men	M	14.6 (13.4–16.8)	29	80.5 ± 7.6 (19.6–2 0)	T_1			26	49.6 ± 6.4 (10.8–174.4)	F_1			58.8 ± 2.8 (32–88)
...ntrol women Non-menstr	F	13.6 (11.7–16.0)	11	52.6 ± 8.9 (11.9–106.6)	T_2			10	34.2 ± 6.0 (11.1–64.3)	F_2			63.5 ± 9.9 (46–79)
...Menstr	Fm	13.2 (11.6–15.3)	13	29.3 ± 6.4 (4.0–83.2)	T_3			10	20.5 ± 4.7 (4.3–38.4)	F_3			70.2 ± 5.6 (33–100)
...n deficiency ...mia	M	12.1 (11.5–13.0)	6	5.5 ± 1.3 (2.7–10.0)	T_4	3.64 (T_3-T_4)	< 0.001	4	2.0 ± 0.4 (1.2–3.0)	F_4	3.92 (F_3-F_4)	< 0.01	51 ± 9.2 (34–74)
...ent hemorrhage ...anemia	M	15.2 (13.5–17.0)	9	30.9 ± 9.0 (7.3– [illegible])	T_5	4.21 (T_1-T_5)	< 0.001	8	14.4 ± 5.1 (0.6–43.4)	F_5	4.32 (F_1-F_5)	< 0.001	40 ± 6.5 (4–58)
...hemorrhage ...anemia	M	14.0 (13.6–14.7)	5	66.5 ± 38.6 (13.4–219.4)	T_6	< 1 (T_1-T_6)	> 0.10	5	32.9 ± 18.1 (7.3–103.3)	F_6	< 1 (F_1-F_6)	~ 0.10	51 ± 6.5 (3–72)
...hemorrhage No ...nia, non-menstr	F	12.7 (11.3–14.7)	5	24.0 ± 8.0 (4.8–42.4)	T_7	2.39 (T_2-T_7)	< 0.05	4	14.7 ± 5.7 (2.3–28.1)	F_7	2.36 (F_2-F_7)	< 0.05	63 ± 6.8 (43–72)
...ory of hemorrhage ...anemia, menstrual	Fm	12.0 (11–12.2)	4	9.7 ± 2.9 (2.4–15.0)	T_8	2.79 (T_3-T_8)	< 0.01	3	5.3 ± 1.1 (1.0–6.5)	F_8	3.15 (F_3-F_8)	< 0.01	44 ± 2.7 (40–49)
...-Poly	M	13.2 (11.1–15.1)	8	21.7 ± 3.2 (4.0–7 5)	T_9	1.94 (T_1-T_9)	0.10 > P > 0.05	6	13.6 ± 6.2 (1.9–41.0)	F_9	2.23 $(F_{10}-F_9)$	0.10 > P > 0.05	56 ± 2.3 (48–65)
...Billroth I	M	14.1 (12.8–15.1)	4	43.4 ± 11.8 (18.8–75.4)	T_{10}	2.50 (T_1-T_{10})	< 0.01	3	37.6 ± 8.8 (28.1–55.3)	F_{10}	1.12 (F_1-F_{10})	~ 0.10	69 ± 3.8 (61– 3)

...= Menstruating women. [a]The means tested are in parentheses.

Bone marrow

This study was performed on 4 groups of subjects

CHRONIC IRON DEFICIENCY ANEMIA. — The group comprised 17 men and 3 women Twelve of the men had undergone Polya gastrectomy most of them 4—7 years before the investigation Seven of the others had a history of hemorrhage. The mean hemoglobin levels for the men was 10.7 and for the women 9 1 g/100 ml The men and women were treated as one group

BLOOD DONORS. — The group comprised 27 male subjects who had for many years been giving one unit of about 450 ml of blood regularly every 2 months. The mean total for each subject was 40 units (range 13—87) During each of the last two years before examination all had given at least 5 blood units per year None of them had received supplementary iron In all but one subject the sternal puncture was performed 5—6 weeks after the last blood donation All blood donors had a hemoglobin concentration of at least 13 5 g per 100 ml mean 14 3 ± 0 1 The mean hemoglobin level obtained for a group of 34 blood donors that had received ferrous iron for 2 months was 14 5 ± 0 1 g per 100 ml

HISTORY OF HEMORRHAGE. — This group was composed of 17 men and 6 women Nine of the men were anemic, having a mean hemoglobin concentration of 10.3 g/100 ml (6.7—13 0) for the others the mean was 14 4 g (13 6—14.7) Three of the women had anemia (range 8.7—10.3)

POLYA PARTIAL GASTRECTOMY — The group comprises 46 men taken at random from the files of Surgical Department I and called for examination to the Out Patients Department. None had had a history of hemorrhage either before or after the gastrectomy and none had had a recurrence of peptic ulcer All were working and only a few had had major complaints after operation. The diet of these subjects appeared on interrogation not to differ from the average diet of the working population except that most of them avoided milk, sweet foods and heavy meals. The mean hemoglobin concentration for the group was 14.3 ± 0 1 g/100 ml (range 13 6—16.0)

Results

IRON DEFICIENCY ANEMIA — The mean of 2.5 μg Fe/mg DNA for the subjects with iron deficiency anemia was significantly lower than the respective means for the three control groups. The upper limit of the observed range was 7.7 μg Fe/mg DNA and the scatter of values for the group was low There was no overlapping between the iron deficiency anemia and the male control group but there was with both groups of control women (Fig 11)

BLOOD DONORS. — There were relatively small differences between the individual values of the group The mean was 4 9 ± 0 4 μg Fe/mg DNA This mean was significantly higher than for the group with chronic iron deficiency anemia ($P < 0.001$) The values for the

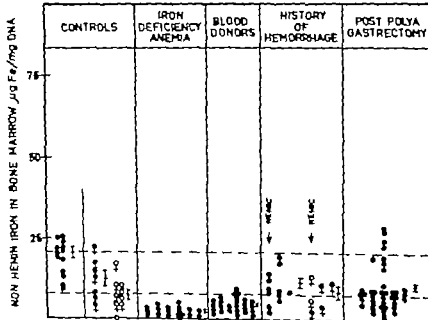


Fig. 11 Non-hemin iron concentrations in bone marrow in states of iron depletion. ● males non-menstruating women menstruating women ![mean ± SE symbol] indicates the mean value with one standard error of the mean. Upper horizontal broken line is drawn through the mean for control men and the lower broken line through the upper limit of iron deficiency anemia

TABLE 21 *Material for study of total non-hemin iron concentration in marrow aspirates in states of iron depletion*

Group	Sex	Hemoglobin (g per 100 ml)		Total non-hemin iron in marrow (µg Fe/mg DNA)		Test of means* $\lvert t \rvert$	P_t
Control men	M	14.7 (13.5–16.2)	15	20.6 ± 1.8 (10.1–36.9)	$\bar{x}_1$		
Control women							
Non menstr	F	13.1 (11.7–16.0)	7	12.5 ± 2.4 (4.4–22.5)	$\bar{x}_2$		
Menstr	Fm	13.1 (11.6–15.3)	9	7.5 ± 1.6 (4.3–21.7)	$\bar{x}_3$		
Iron deficiency anemia	M+F	10.3 (5.9–12.0)	20	2.5 ± 0.4 (0.0–7.7)	$\bar{x}$	3.04 ($x - \bar{x}_4$)	
Blood donors	M	14.3 (13.6–15.2)	27	4.9 ± 0.4 (2.5–9.9)	$\bar{x}$	4.2 ($\bar{x}_1 - \bar{x}_4$)	< 0.001
History of hemorrhage	M	12.3 (8.7–14.7)	17	11.4 ± 1.5 (1.9–22.4)	$\bar{x}$	3.92 ($\bar{x}_1 - \bar{x}_4$)	< 0.001
	F	10.9 (8.7–11.9)	6	8.7 ± 2.0 (2.9–14.3)	$\bar{x}_7$	1.22 ($\bar{x}_2 - \bar{x}$)	~ 0.10
Post-Polya gastrectomy	M	14.3 (13.6–16.0)	46	10.6 ± 1.1 (1.4–31.5)	$\bar{x}$	4.74 ($\bar{x}_1 - \bar{x}_8$)	< 0.001

m = Menstruating women *The means tested are in parentheses

Bone marrow

This study was performed on 4 groups of subjects.

CHRONIC IRON DEFICIENCY ANEMIA. — The group comprised 17 men and 3 women. Twelve of the men had undergone Polya gastrectomy most of them 4—7 years before the investigation. Seven of the others had a history of hemorrhage. The mean hemoglobin levels for the men was 10.7 and for the women 9.1 g/100 ml. The men and women were treated as one group.

BLOOD DONORS. — The group comprised 27 male subjects who had for many years been giving one unit of about 450 ml of blood regularly every 2 months. The mean total for each subject was 40 units (range 13—87). During each of the last two years before examination all had given at least 5 blood units per year. None of them had received supplementary iron. In all but one subject the sternal puncture was performed 5—6 weeks after the last blood donation. All blood donors had a hemoglobin concentration of at least 13.5 g per 100 ml, mean 14.3 ± 0.1. The mean hemoglobin level obtained for a group of 34 blood donors that had received ferrous iron for 2 months was 14.5 ± 0.1 g per 100 ml.

HISTORY OF HEMORRHAGE. — This group was composed of 17 men and 6 women. Nine of the men were anemic, having a mean hemoglobin concentration of 10.3 g/100 ml (6.7—13.0); for the others the mean was 14.4 g (13.6—14.7). Three of the women had anemia (range 8.7—10.3).

POLYA PARTIAL GASTRECTOMY. — The group comprises 46 men, taken at random from the files of Surgical Department I, and called for examination to the Out Patients Department. None had had a history of hemorrhage either before or after the gastrectomy and none had had a recurrence of peptic ulcer. All were working and only a few had had major complaints after operation. The diet of these subjects appeared on interrogation not to differ from the average diet of the working population except that most of them avoided milk, sweet foods and heavy meals. The mean hemoglobin concentration for the group was 14.3 ± 0.1 g/100 ml (range 13.6—16.0).

Results

IRON DEFICIENCY ANEMIA. — The mean of 2.5 µg Fe/mg DNA for the subjects with iron deficiency anemia was significantly lower than the respective means for the three control groups. The upper limit of the observed range was 7.7 µg Fe/mg DNA and the scatter of values for the group was low. There was no overlapping between the iron deficiency anemia and the male control group but there was with both groups of control women (Fig. 11).

BLOOD DONORS. — There were relatively small differences between the individual values of the group. The mean was 4.9 ± 0.4 µg Fe/mg DNA. This mean was significantly higher than for the group with chronic iron deficiency anemia ($P < 0.001$). The values for the

nics, however the last stages of progressive iron depletion cannot be followed because the stainable hemosiderin disappears before the non-hemin iron is exhausted [124] It is the last stage of iron depletion before anemia appears that is of particular interest, since it is then that any changes in serum iron, iron-binding capacity and intestinal iron absorption would be expected to occur In the cases of mild iron deficiency anemia of the present study the mean non-hemin iron concentration in the liver was 5.5 mg per 100 g of dry weight (range 2.7—10.0) These values are comparable with those found by Hallgren in rats with severe iron deficiency anemia. No overlapping was observed between the subjects with iron deficiency anemia and the male and post-menopausal female control groups. There was however an overlapping between the iron deficiency anemia group and the control group of menstruating women. Some of the non-anemic subjects with a history of hemorrhage and those after Polya partial gastrectomy had iron concentrations in the liver within the range of iron deficiency anemia.

Analyses of bone marrow in which larger and more homogeneous groups of iron-deficient subjects were examined, gave similar findings. There was no overlapping of values between the group of iron deficiency anemia and the male controls, but there were a large number of non-anemic cases, particularly among the post Polya patients and the blood donors, the values for whom were indistinguishable from those for cases of iron deficiency anemia. These findings substantiate the view that the storage iron must be used up before iron deficiency anemia ensues and that this critical value would not necessarily be lowered when anemia develops. Here, then, is a state of iron depletion without anemia.

Non-anemic patients with a history of hemorrhage had significantly lower mean non-hemin iron concentrations than their respective control groups. A subdivision of the non-anemic men into those who had had recent hemorrhage and those who had had hemorrhage at least one year earlier did not reveal significant differences. This is in accordance with the view that iron stores, once depleted, recover very slowly [103 95]

The blood donors are of particular interest for they were a homogeneous group as regards iron stores, which, because of the frequent and regular phlebotomies without supplementary supply of iron, must have been exhausted. If the term "iron deficiency anemia" denotes the state in which there is not enough iron in the body to provide a normal hemoglobin level [96] such blood donors would have a true iron deficiency anemia after every phlebotomy The iron required to regenerate the hemoglobin must be obtained from exogenous sources. At the time of the examination — 6 weeks after the last phlebotomy — they had regenerated about 58 g of hemoglobin, which will have necessitated a daily uptake of about 4 mg of iron from food in excess of losses. This is in agreement with figures given for the maximum uptake of iron from food by iron-deficient subjects [125 96] The mean non-hemin

blood donors were significantly lower than those for the control men and non-menstruating women but not for menstruating women

History of hemorrhage. — Since the means for the anemic and non anemic men were not significantly different (10.7 ± 2 0 and 12.2 ± 2.3 μg Fe/mg DNA respectively) they were considered as a single group The means for this and both sub-groups were significantly lower than the mean for the male control group

The mean for the 6 women was not significantly different from those for either group of control women.

Polya partial gastrectomy — The mean non hemin iron in bone marrow for the gastrectomy group was significantly lower than that for male controls. For 15 of the subjects, or 33 % of the group the value was less than 7 6 μg Fe/mg DNA, that is, within the range of iron deficiency anemia For 26 of the subjects, or 57 % of the group the values were below the lowest for the male controls.

Discussion

Abnormal blood loss is the most frequent cause of negative iron balance. A daily loss exceeding 6—8 ml of blood equivalent to 3—4 mg of iron, will usually result in a negative balance, since this is the maximum amount of iron that can be absorbed from an ordinary diet by the iron depleted subject [109 103] The second cause of a negative iron balance is a low uptake from the gut because of either too low an intake of iron or impairement of the absorptive capacity of the intestinal wall the latter is the case in idiopathic steatorrhea [110 111] A more common cause of inefficient iron absorption from foods in Western countries is partial gastrectomy and especially by the Polya operation [112—115]

When a negative iron balance persists because of continued loss of blood or reduced absorption, the iron stores gradually diminish. Hemosiderin and ferritin iron are mobilized for erythropoiesis [116] and before iron deficiency anemia develops both fractions are almost consumed [50 103 117—120] Studying the quantitative changes in non-hemin iron in the rat during different degrees of induced negative iron balance Hallgren [50] found that the degree of anemia in nutritional iron deficiency depends on the degree of iron restriction in the diet. However the absence of any difference between the animals with mild and severe anemia as regards the non hemin iron concentration in the liver and spleen indicates that once a condition of iron deficiency anemia has developed the iron store is exhausted and further restriction of iron supply will result only in a diminution of the total hemoglobin The liver of rats with iron deficiency anemia in Hallgren's experiments contained 0 9 mg of non hemin iron per 100 g of fresh tissue, i.e. about 3 0 mg per 100 g dry weight.

The systematic study of iron stores in states of iron depletion in man has relied mainly on semiquantitative histochemical methods [118—123] With these tech-

the control mean for 57 % they were below the minimum for the controls, and for 33 % they were within the range of iron deficiency anemia. It is outside the scope of the present study to discuss in detail the problem of gastrectomy and iron absorption from food, but it may be mentioned that the analysis of the findings of another investigation has disclosed a progressive fall in non-hemin iron and a parallel rise in the total iron-binding capacity with time after the Polya operation. The regression on time after the operation was significant even for the non-anemic and non-hemorrhagic men [127]

iron concentration in the bone marrow of the blood donors, although low was significantly higher than the mean for the subjects with iron deficiency anemia (Table 21) with a difference of 2 4 μg Fe/mg DNA Assuming a mean total red marrow weight of 1500 g [126] and a mean DNA concentration of 450 mg per 100 g of red marrow the non hemin iron content of the total red marrow of the blood donors would be approximately about 33 mg (6750 × 0 0049) Since the red marrow volume and DNA content for the blood donors are probably considerably higher and those for the subjects with non hemorrhagic iron deficiency anemia, lower than for normal individuals, it is reasonable to assume a total of 9000 mg of DNA for the donors and 4500 mg for chronic iron deficiency anemia The corresponding figures for the non hemin iron content would then be 44 1 mg (9000 × 0 0049) and 11.2 mg (4500 × 0 0025) On these rough assumptions, the blood donors who had just recovered from iron deficiency anemia would have an excess of about 32.9 mg non hemin iron in the red marrow as compared with the group of iron deficiency anemia.

The donors differed in two other respects from this group Their serum iron concentration was 106 ± 9 7 μg against 43 ± 5 7 μg/100 ml and they had a mean sideroblast count in the bone marrow smears of 34 per cent, against 2 per cent in the iron deficiency anemia group. The sideroblasts normalize rapidly when a small amount of iron in excess of the hemoglobin requirements is administered to an iron deficient person [124] It would therefore seem that the excess of about 32 9 mg non hemin iron present in the total red marrow of the blood donors constitutes the sideroblast pool of these subjects. Since their mean sideroblast count was about 25 % below normal the pool of non hemin iron in the bone marrow for a normal sideroblast count would be about 44 1 mg (32.9/0.75) or only 33 1 mg in excess of iron deficiency anemia assuming that the normal marrow DNA content is three-quarters of that of the donors. Since the sideroblasts of blood donors who have just recovered from anemia usually contain fewer and smaller granules than in the normal the above estimates of the sideroblast iron pool for the normal subject indicate only minimum values.

The non-hemin iron level in the liver was significantly lower for the men without a history of hemorrhage who had undergone Polya or Billroth I partial gastrectomy than for the male control group The values were lowest for the Polya patients, but the difference in the means for this and the Billroth I group was only almost significant While it must be borne in mind that the series was small the findings are consistent with the conclusion reached in earlier investigations — namely that iron deficiency anemia is more common after the Polya operation especially in women [114 115] Determinations of non hemin iron in the bone marrow in a series of non anemic, non hemorrhagic men that had undergone a Polya operation have shown that for about 90 % of the Polya patients the values were below

The eighth subject had an uncomplicated gastric ulcer with anemia that was not due to iron deficiency

Results

MEN WITHOUT ANEMIA. — The mean value of 82.4 mg per 100 g for the total non-hemin iron in the liver and of 40.6 mg for ferritin iron were not significantly different from the means for the male control group Nor was there any statistical difference as regards the per centage of ferritin iron.

MEN WITH ANEMIA. — The mean value for the total non-hemin iron was 112 mg per 100 g and that for ferritin iron 66.6 mg. While these means are higher than those for the control group the difference was not significant. The dif ference in the means of storage iron concentrations might be due to the dif ferences in the mean hemoglobin con- centration in the anemic and control groups. The mean hemoglobin concentra tion was 2.5 g per 100 ml lower than for the male control group

The percentage of ferritin iron did not differ from that of the controls.

WOMEN WITH ANEMIA. — The mean non-hemin iron concentration was 35.7 mg/100 g and the mean ferritin iron concentration 23.5 mg. These values were lower than those for the corres- ponding control group but not signi- ficantly so.

The percentage of ferritin iron did not differ from that for the controls.

In summary: for none of the 3 ana lyzed groups did the means differ significantly from those of the controls in respect of total non-hemin and fer ritin iron or of the percentage of fer ritin iron. Only one patient in the male group with anemia of infection had a value above the range of the controls.

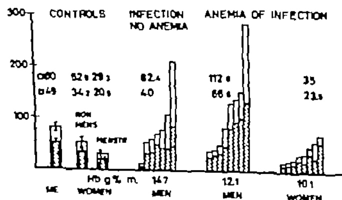


Fig. 12 Total non-hemin iron (whole bars) and fer ritin iron (hatched bars) con- centrations in liver biopsy specimens of subjects with infection or malignant di sease without and with anemia. The figures are the means for the group.

Total Non-Hemin and Ferritin Iron in Infectious and Malignant Disease

Unless otherwise stated the cases of infectious or toxic disease in this study had no history of hemorrhage, gastro-intestinal tumours or callous or penetrating gastric ulcers nor had they received blood transfusions, injections of iron or oral iron therapy over more than one month

Liver

Storage iron in liver tissue was examined in anemic and non anemic men and anemic women

The male group without anemia consisted of (i) 4 cases of chronic cholecystopathy with fever complicated by jaundice in 3 and colitis in one instance, and (ii) 3 cases of uncomplicated gastric ulcer and hepatic disease 2 of them had portal cirrhosis with distortion of the liver architecture (nos. 53 54[1]) and the third (no 55) had a moderate augmentation of periportal connective tissue with infiltration of round cells this subject had had epidemic hepatitis about 20 years previously he had the largest amount of iron in this group and was judged by the pathologist to be hemosiderotic.

The group with anemia comprised 8 men and 8 non-menstruating women. Six of the males had a clinical diagnosis of chronic and acute cholecystopathy with fever this was complicated in 2 instances by jaundice and acute pancreatitis, in one by jaundice and biliary cirrhosis, one by jaundice, one by pernicious anemia in relapse, and one by mediastinal lymphadenosis and chronic subfebrility The other 2 subjects were a case of gastric stenosis with anemia of infection and one of abdominal cancer probably of pancreatic origin with metastases (no 61) This patient had the highest iron content encountered in the whole material Histologic examination disclosed moderate steatosis but no augmentation of connective tissue or changes in liver structure. The Prussian blue reaction showed grade 3 of iron in the parenchymal cells and grade 1 in the histiocytes.

Of the 8 post-menopausal women with anemia 7 had chronic gall bladder disease, complicated in one case each by a bile fistula, a sterile abscess in a liver cyst, rheumatoid arthritis, subacute pyelonephritis, jaundice with incipient biliary cirrhosis, and recurrent subacute cholecystopathy with infiltration of lymphocytes and histiocytes in the liver

[1] See *Appendix*

Bone marrow

Storage iron in the bone marrow was examined in male and female cases of infectious diseases with and without anemia. Another two groups comprised subjects with megaloblastic anemia and subjects with anemia and exogenous iron load in which men and women were treated as a single group.

Of the 11 men without anemia one had regional ileitis, one bronchopneumonia and venous thrombosis, one active alcoholic cirrhosis of the liver with jaundice, one gastric ulcer with inactive chronic hepatitis and 6 chronic cholecystopathy in 2 instances complicated by jaundice and cholangitis.

Of the 5 women without anemia *2 were menstruating*. All had subacute cholecystopathy in 3 instances complicated by jaundice and pancreatitis.

Of the 8 men with anemia 2 had active cirrhosis of the liver 2 subacute cholecystopathy and cholangiohepatitis, 1 carcinoma of the pancreas with metastases and another chronic subfebrility with stomatitis.

The 8 women with anemia were *all non-menstruating*. Two had rheumatoid arthritis, one active alcohol cirrhosis of the liver one subacute sigmoiditis with abscess formation, one uncomplicated gastric ulcer with infection, one refractory anemia, one myelomatosis and one acute myeloic leukemia.

A group of megaloblastic anemias in relapse comprised 5 male and 4 female subjects.

A miscellaneous group consisted of 4 men and 8 women with anemia of infection, malignant tumour and refractory anemia, all had received parenteral iron or blood transfusions.

Results

Of the six groups studied only the group of megaloblastic anemia and the group of miscellanous anemia with exogenous load of iron had significantly higher means than the controls.

Within the megaloblastic group, who had the highest degree of anemia, there was no difference between the mean non-hemin iron value for the men and women, and the individual values of these two subgroups were within the same normal range (Fig 13) In the miscellanous group however with its considerably higher mean hemoglobin value the scatter for the women was greater one of them had a value within the range of iron deficiency anemia (Fig. 13)

The 4 other groups of men and women with and without anemia had slightly higher means than the respective control groups, but not significantly so. As the small group of non-anemic women was composed of both menstruating and non menstruating subjects it was tested against menstruating controls, but even so the difference was not significant. The hemoglobin level of the anemic groups was only moderately lowered, and the anemia in the groups of men and women was of a comparable degree.

Values above the range of the respective control groups were recorded as follows: among the men, one case of gastric ulcer with inactive chronic hepatitis (histological diagnosis), who also

TABLE 22. *M terial f study of total non-hemin iron and ferritin i on in liver biopsy pecimens in states of infection and t xicity*

Group	Sex	Hemoglobin (g/100 ml)		Total non-hem iron in liver $\bar{T}$ (mg % dry wt)		Test of means[a] $\|t\|$	P		Ferritin i on in li er $\bar{f}$ (mg % dry wt)		Test of means[a] $\|t\|$	P
Control men	M	14 6 *(13.5–16.8)*	29	80.5±7 6 *(19.0–2 7 0)*	$\bar{T}_1$			26	49 8±6 4 *(10.8–1 4 4)*	$\bar{f}_1$		
Control women												
Non-menstr.	F	13 6 *(11 7–16.0)*	11	52.6±8.9 *(11.9–106.6)*	$\bar{T}_2$			10	34 2±6.0 *(11 1–64 3)*	$\bar{f}_2$		
Menstr	Fm	13.2 *(11.6–15 3)*	13	29.3±6.4 *(4 0–83.2)*	$\bar{T}_3$			10	20 5±4 7 *(4 5–58 4)*	$\bar{f}_3$		
Infection. No anemia	M	14 7 *(13.5–17 7)*	7	82.4±23.3 *(10 7–206 5)*	$\bar{X}_4$	<1 $(\bar{T}_4-\bar{X}_1)$	0 10	7	40 6±8 8 *(6 –84 0)*	$\bar{f}_4$	1 $(\bar{f}_1-\bar{f}_4)$	0 10
Anemia of infection and toxicity	M	12.1 *(10.5–13.3)*	8	112.0±28.7 *(36 1–279.0)*	$\bar{X}_5$	1 06 $(\bar{X}_5-\bar{T}_1)$	>0 10	8	66 6±14 0 *(23 8–130 8)*	$\bar{f}$	1.09 $(\bar{f}_5-\bar{f}_1)$	0 10
Anemia of infection nd toxicity	F	10.1 *(8.3–11 4)*	8	35 7±6.5 *(16 4–50.6)*	$\bar{X}_6$	1 53 $(\bar{T}_2-\bar{X}_6)$	0.10		23 5±5.2 *(11 3–54 9)*	$\bar{f}_6$	1.35 $(\bar{f}_2-\bar{f}_6)$	>0.10

Fm = Menstruating women [a]The means tested are in parentheses

had a high iron level in the liver (no. 55) one case of regional ileitis (no. 258) and one of cholangitis and severe jaundice (no. 67); among the women, one with jaundice and no anemia (no. 132) and one with sigmoiditis and anemia (no 273)

Discussion

The effect of infection and neoplastic disease on the erythron and on iron metabolism in particular has received much attention during the last two decades [128—130] Hypoferremia, lowered plasma iron binding capacity [88] increased plasma iron clearance and plasma iron turnover [134 137 138] are typical of both infectious and neoplastic diseases. The anemia has been attributed to a moderately shortened erythrocyte life span and an inability of the marrow to compensate for it. The anemia of infection and malignant disease is usually mild and occasionally moderately severe. The degree of anemia is related to the severity and duration of infection [129] Mild, short lived infections do not necessarily cause anemia. If anemia appears iron is diverted from the hemoglobin compartment and deposited in the storage organs. Thus the increase in storage iron would be proportional to the degree of anemia developed. In the absence of anemia the iron stores would remain constant provided that there is no increase in iron absorption during the course of the infection The problem of iron absorption during the course of infectious disease is, however a matter of disagreement. [135 137—143] Heilmeyer and his co-workers suggested that during infection there is hyperactivity of the reticulo-endothelial system in consequence of which a larger amount of iron is required thus is accumulated in the RES in the form of hemosiderin and it participates in detoxification and combatting of infection. As a result of this increase in the iron requirements the absorption of iron from the gut is enhanced. [137 144] However an increase in absorption of iron from foods in infectious disease would have to continue a long time to produce a significant rise in the storage iron level. Moreover the usual reduction in food intake in cases of chronic infections would tend to counterbalance any increase in absorption. Hence the main factor responsible for an augmentation of the iron stores in infection and malignant disease would be the associated secondary anemia. The ultimate size of the iron stores in the individual case will be the sum of the previous stores and the iron derived from the destroyed hemoglobin mass. Since the former iron stores of these subjects are dependent on factors influencing the iron balance, account must be taken of these factors in any meaningful comparison of the pathological cases with controls.

The results of the present study are consistent with the above reasoning. Thus, the male group without anemia did not differ from the male control group as regards the non-hemin and ferritin iron content in the liver tissue.

Case 55 deserves special comment. He was admitted for an uncomplicated gastric ulcer and 20 years previously

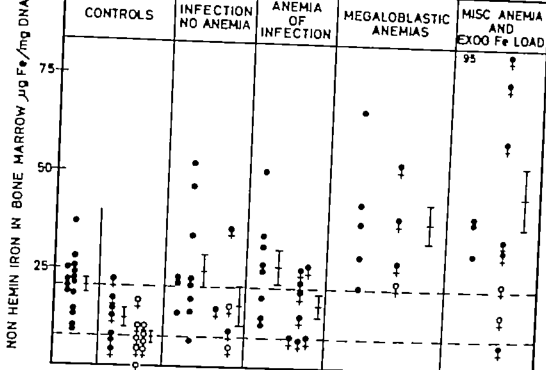


Fig 13 Total non hemin iron in marrow aspirates of controls and cases of infectious or toxic disease. Symbols as in Fig. 11 The upper broken horizontal line indicates the mean for the control males; the lower line indicates the upper limit of iron deficiency anemia.

TABLE 23 *Material for study of total non-hemin iron concentration in bone marrow aspirates in states of infection and toxicity*

Group	Sex	Hemoglobin (g per 100 ml)		Total non-hemin iron in marrow (μg Fe/mg DNA)	Test of means[a] $\lvert t \rvert$	P_t
Control men	M		15	20.6 ± 1.8 $\bar{x}_1$ (10.1—36.9)		
Control women						
Non-menstr	F		7	12.5 ± 2.4 $\bar{x}_2$ (4.4—22.5)		
Menstr	Fm		9	7.5 ± 1.6 $\bar{x}_3$ (4.3 [illegible])		
Infection. No anemia	M	14.6 (13.5—17.7)	10	25.9 ± 4.5 $\bar{x}_4$ (7.0—52.7)	1 ($\bar{x}_4 - \bar{x}_1$)	> 0.10
Anemia of infection and toxicity	M	11.0 (5.4—13.3)	8	24.9 ± 4.8 $\bar{x}$ (11.5 50.6)	< 1 ($\bar{x} - \bar{x}_1$)	0.10
Infection. No anemia	F	13.3 (11.7—14.6)	5	16.1 ± 5.2 $\bar{x}$ (5.0—35.3)	1.58 ($\bar{x}$ $\bar{x}_1$)	0.10
Anemia of infection and toxicity	F	9.1 (6.3—10.1)	8	16.3 ± 2.9 $\bar{x}_7$ (7.3—26.4)	1.01 ($\bar{x} - \bar{x}_2$)	> 0.10
Megaloblastic anemia	M+F	6.9 (4.5—11.9)	9	38.0 ± 5.0 $\bar{x}_8$ (15—66.8)	3.27 ($\bar{x}_8 - \bar{x}_1$)	< 0.01
Miscellaneous anemia and exogenous iron load	M+F	9.6 (5.1—12)	1	44.9 8.1 $\bar{x}_9$ (6.6—94.9)	2.9 ($\bar{x}_9 - \bar{x}_1$)	0.01

had a high iron level in the liver (no. 55), one case of regional ileitis (no. 258) and one of cholangitis and severe jaundice (no 67) among the women, one with jaundice and no anemia (no. 132) and one with sigmoiditis and anemia (no 273)

Discussion

The effect of infection and neoplastic disease on the erythron and on iron metabolism in particular has received much attention during the last two decades [128—130] Hypoferremia, lowered plasma iron binding capacity [88] increased plasma iron clearance and plasma iron turnover [134 137 138] are typical of both infectious and neoplastic diseases. The anemia has been attributed to a moderately shortened erythrocyte life span and an inability of the marrow to compensate for it. The anemia of infection and malignant disease is usually mild and occasionally moderately severe. The degree of anemia is related to the severity and duration of infection [129] Mild, short-lived infections do not necessarily cause anemia. If anemia appears iron is diverted from the hemoglobin compartment and deposited in the storage organs. Thus the increase in storage iron would be proportional to the degree of anemia developed. In the absence of anemia the iron stores would remain constant provided that there is no increase in iron absorption during the course of the infection The problem of iron absorption during the course of infectious disease is, however a matter of disagreement. [135 137—143] Heilmeyer and his coworkers suggested that during infection there is hyperactivity of the reticuloendothelial system in consequence of which a larger amount of iron is required this is accumulated in the RES in the form of hemosiderin and it participates in detoxification and combatting of infection. As a result of this increase in the iron requirements the absorption of iron from the gut is enhanced. [137 144] However an increase in absorption of iron from foods in infectious disease would have to continue a long time to produce a significant rise in the storage iron level. Moreover the usual reduction in food intake in cases of chronic infections would tend to counterbalance any increase in absorption. Hence the main factor responsible for an augmentation of the iron stores in infection and malignant disease would be the associated secondary anemia. The ultimate size of the iron stores in the individual case will be the sum of the previous stores and the iron derived from the destroyed hemoglobin mass. Since the former iron stores of these subjects are dependent on factors influencing the iron balance, account must be taken of these factors in any meaningful comparison of the pathological cases with controls.

The results of the present study are consistent with the above reasoning Thus, the male group without anemia did not differ from the male control group as regards the non-hemin and ferritin iron content in the liver tissue.

Case 55 deserves special comment. He was admitted for an uncomplicated gastric ulcer and 20 years previously

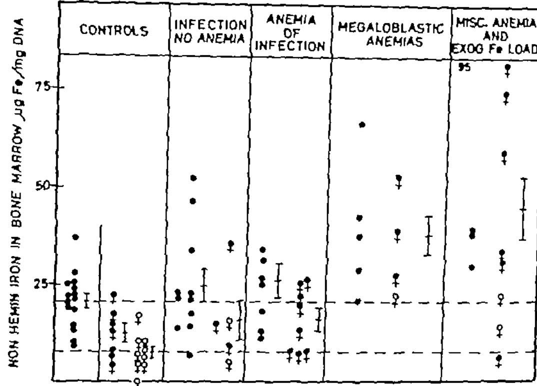


Fig 13 Total non-hemin iron in marrow aspirates of controls and cases of infectious or toxic disease. Symbols as in Fig 11 The upper broken horizontal line indicates the mean for the control males the lower line indicates the upper limit of iron deficiency anemia.

TABLE 23 *Material for study of total non-hemin iron concentration in bone marrow aspirates in states of infection and toxicity*

Group	Sex	Hemoglobin (*g per 100 ml*)	*n*	Total non-hemin iron in marrow (*µg Fe/mg DNA*)		Test of means* $\|t\|$	P_t
Control men	M		15	20.6±1.8 (10.1–36.9)	$\bar{x}_1$		
Control women							
Non-menstr	F		7	12.5±2.4 (4.4–22.5)	$\bar{x}$		
Menstr	Fm		9	7.5±1.6 (4.3–23.7)	$\bar{x}_3$		
Infection. No anemia	M	14.6 (13.5–17.7)	10	25.9±4.3 (7.0–52.7)	$\bar{x}_4$	< 1 ($\bar{x}_4-\bar{x}_1$)	0.10
Anemia of infection and toxicity	M	11.0 (5.4–13.3)	8	24.9±4.8 (11.5–50.6)	$\bar{x}_5$	< 1 ($\bar{x}-\bar{x}_1$)	0.10
Infection. No anemia	F	13.3 (11.7–14.6)	5	16.1±5.2 (5.0–35.3)	$\bar{x}$	1.58 ($\bar{x}-\bar{x}_3$)	0.10
Anemia of infection and toxicity	F	9.1 (6.3–10.1)	8	16.3±2.9 (7.3–26.4)	$\bar{x}_7$	1.01 ($\bar{x}_7-\bar{x}_2$)	> 0.10
Megaloblastic anemia	M+F	6.9 (4.5–11.9)	9	38.0±5.0 (21.5–66.8)	$\bar{x}_8$	3.27 ($\bar{x}_8-\bar{x}_1$)	0.01
Miscellaneous anemia and	M+F	9.6 (5.1–13.7)	1	44.9±8.1 (6.6–94.9)	$\bar{x}_9$	2.92 ($\bar{x}-\bar{x}_1$)	0.01

rheumatic fever but there was a statistically significant increase for the spleen and for histochemically stainable iron in the Kupffer cells of the liver though not for the parenchymal cells. Hallgren found high values for non-hemin iron concentration in the liver in cases of non-hemorrhagic malignant tumours and infectious disease with anemia but in none of these cases was the increase deemed to be so great as not to be ascribable to the reduction in the total hemoglobin mass [50] A large autopsy material of cases of infection and malignant tumours published by Morgan and Walters [79] showed no statistically higher total non-hemin iron concentration in the liver but there was a significant increase in the spleen. As the main object of these authors was to study the distribution of ferritin their material was not grouped with respect to sex and menstruation and they give no data on the degree of anemia.

The mild anemia in our series may account for the absence of any statistically significant increase in non-hemin iron. For the cases of megaloblastic anemia who had a greatly lowered hemoglobin concentration, however the mean non hemin iron concentration in the marrow was significantly higher It is estimated that these patients increased their stores by about 1000 mg of iron which was derived from destroyed hemoglobin.

The highest values of non-hemin iron in bone marrow were encountered in cases of infection and anemia who received an exogenous load of iron. However the results have also shown that even in this group there were women with critically low non-hemin iron values (Fig. 13) a fairly low non-hemin iron value is not uncommon in anemia of infection — for instance, women with rheumatoid arthritis who take large amounts of salicylates and in cases of pyelonephritis and uremia. Although iron deficiency is by definition not the limiting factor for anemia of infection, it may accompany the disease, and when the infection is abolished it may be necessary to give iron to normalize the hemoglobin level Within the last group the highest values recorded for the bone marrow 95 and 85 µg Fe/mg DNA, related to a case of refractory anemia and transfusional hemosiderosis (no. 289) and a female alcoholic with hypoproteinemia and cachexia (no. 271). The last patient had for many years consumed large amounts of French wine and a French aperitif [148] Her urinary excretion of xanthurenic acid after tryptophan loading was high and became normal after administration of pyridoxin. She had severe anemia and had previously had a 3-month course of oral iron therapy These measures alone would hardly account for the large accumulation of iron in the marrow encountered, and there would therefore seem to have been an increase in the absorption of iron from the gut. It may also be noted that only 27% of the total non-hemin iron was present as ferritin It can however be inferred that the high values obtained in this case were due to a reduction in DNA concentration and volume of the cellular marrow In

had had epidemic hepatitis. The histological examination of the liver disclosed an augmentation of connective tissue and round-cell infiltration of the periportal fields. The Prussian blue reaction showed grade 3—4 of hemosiderin in the parenchymal cells and grade 2 in the Kupffer cells. The level of total non hemin iron was 206 mg/100 g, dry weight. Such a condition is usually regarded as liver hemosiderosis, and since there was some fibrosis of the periportal fields a causal relationship between the two findings might be suspected There is, however no basis for such a conclusion since case 16 a healthy control with no histological changes in the liver had grade 3 hemosiderin in the parenchymal and Kupffer cells and the total non-hemin iron concentration in liver tissue was 227 mg/100 g dry weight. Although the percentage of ferritin was lower for case 55 its value falls within the range of the control males. As was recently pointed out by MacDonald and Pechet, the interpretation of liver hemosiderosis must be made against a background of control information [145 146] Similar findings were obtained in the analysis of bone marrow The mean non hemin iron concentrations for the men and women with infection but without anemia were not different from those for the respective control groups.

For the male cases of infectious diseases with anemia the non hemin iron concentration in the liver was higher than that for the controls, but not significantly so The mean non hemin iron concentration in liver for this group was 32 mg per cent higher than the mean for the male control group. This difference may be ascribed to a reduction in the hemoglobin mass, which, estimated from the differences in the mean hemoglobin concentration, is about 113 g This represents a release of about 380 mg of iron to the stores. A deposit of one third of this in the liver would augment the iron concentration by about 25 mg/100 g dry weight. In the case of anemic women however the mean liver values were rather lower than but not significantly different from the controls. The bone marrow study gave essentially the same results, in that only the group with severe megaloblastic anemia and those who received an exogenous load of iron had significantly increased storage iron values.

Autopsy studies on patients dying from infectious and malignant diseases have consistently revealed high iron levels only in the spleen [76 77 147] These may be ascribed wholly to the secondary anemia and destruction of hemoglobin The findings relating to liver iron have been inconsistent Gross, Sandberg and Holly [76] found high values in liver in only one third of their malignant cases in several instances high values were recorded even in the absence of anemia. Since no division of the control groups with regard to sex was made the comparison of individual cases with controls is irrelevant. The mean value for their small control group was 45% lower than that for the present male control group Schairer and Rechenberger [147] did not find high values for total non-hemin iron in the liver of patients dying of sepsis, tuberculosis or

Ferritin Iron in Relation to Total Non-Hemin Iron in Basal Subjects and Pathological States

The amounts of ferritin and hemosiderin in various organs and the relationship between them have been studied in animals under different experimental conditions [116, 149—157]. Studies in man have been performed on autopsy specimens by Wöhler *et al.* [158, 159] with the method of Kaiderling *et al.* [61] and by Morgan and Walters [79] with the method of Gabrio *et al.* [62] as modified by Kaldor [63]. The results and conclusions of these studies are inconsistent. In the present study the proportion of ferritin iron in the total non-hemin iron under basal conditions and in pathological states was analysed.

Material

Non-hemin iron and ferritin iron were determined in a total of 126 surgical liver specimens and in 44 bone marrow aspirates. The first group of patients was considered large enough to warrant a statistical analysis. From these patients 55 were selected to provide a basal group with which the pathological groups could be compared. The basal subjects are defined as not having anemia, signs of infection or chronic illness, or a history of hemorrhage during the year before examination — conditions that have been considered to alter the ferritin to hemosiderin ratio [154, 155, 158, 159].

From the remaining 71 pathological cases the following 6 groups were selected.

(*i*) Recent hemorrhage with no signs of infectious disease: 12 subjects. Two had moderate iron deficiency anemia and one acute post-hemorrhagic anemia.

(*ii*) Mild infection without anemia or a history of hemorrhage: 13 subjects (Inf I). All were cases of chronic or acute cholecystitis.

(*iii*) Infectious or toxic disease without anemia or hemorrhage: 8 subjects. Two had liver cirrhosis, one chronic hepatitis and the others cholecystopathy complicated by jaundice or/and liver steatosis. Clinically the infectious disease was moderate to mild (Inf II).

(*iv*) Infectious or toxic anemia with no history of hemorrhage: 16 subjects. In these patients the disease was more active and severe — mainly diseases of the liver and biliary tract, and one case of pancreatic carcinoma with metastases (no. 61).

(*v*)[1] Seven non-basal subjects with a total non-hemin iron concentration in the liver above 100 mg/100 g. dry weight. Five of them had infectious or

general however our results — in common with those of Rechenberger and Schairer [147] — are consistent with the view of Cartwright & Wintrobe [129] and Hallgren [50] that the increase in iron stores in cases of chronic infection with anemia and cases of cachexia is due mainly to a reduction in the hemoglobin and myoglobin mass.

literature that the percentage of ferritin in total non-hemin iron is diminished in infectious disease and after hemorrhage [152, 155 158 159] the following analysis was performed. If it is assumed that the pathological groups are comparable with the basal, any difference between the observed values and those calculated from basal conditions would be due to random variation. The mean differences for each group were therefore examined by the t test and if a mean difference was significant, the hypothesis that the pathological group was equivalent to the basal one was rejected.

For each value of total non-hemin iron (x) in the respective pathological groups the ferritin iron was calculated (y_{calc}) by inserting the value of x in the equation for the basal subjects $\bar{y} = 0.04 + 0.60\ x$. From the differences (d) between the calculated (y_{calc}) and observed (y_{obs}) ferritin values the mean difference ($\bar{d}$) for the group and its standard error ($\sigma_{\bar{d}}$) were determined. The significance of this mean deviation of the respective pathological group from the basal line was then examined by the t test.

As regards the pathological groups the only significant differences from the basals were recorded for the group with recent hemorrhage and the group with mild infections (Inf) (Fig. 15 Table 24)

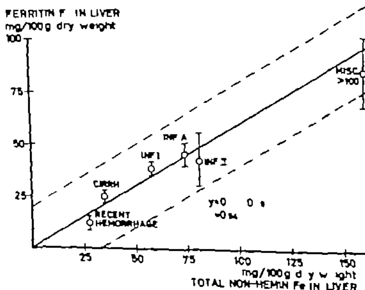


Fig. 13 The relation of ferritin iron to total non-hemin iron in pathological groups compared with the basal group. Linear regression and the 95% population limits for the basal subjects. The circles with the dot denote the mean difference between the observed ferritin values and those calculated from the basal equation. The vertical lines denote ± twice the standard error of the mean deviation. For abbreviations and details of the groups see Table 24

toxic disease of the liver or biliary tract and one had a malignant disease.

(vi)[1] Four cases of liver cirrhosis, 2 of the Laënnec and 2 of the biliary type (nos 53 54 66 74)

Results

BASAL SUBJECTS. — The relationship between ferritin and total non hemin iron in the liver in the basal group is shown in Fig 14 The mean of total non hemin iron for the group was 56 7 and for ferritin iron 34 1 mg/100 g The observed range for total non hemin iron was 4 0—227 0 mg/100 g dry weight. Within the above range of values there was a strong correlation between the ferritin iron and total non hemin iron. Since the constant in the equation of the linear regression was low and not different from zero the mean ferritin iron was 60% of total non-hemin iron.

Since there was reason to suppose that the percentage of ferritin might diminish at high values of total non hemin iron [116 79] the basal subjects were divided into two groups, one with low and *the other with high total non hemin iron* concentrations there was no significant difference between them in respect of the percentage of ferritin. For the higher concentrations of total non hemin iron there was a tendency towards a greater dispersion of the values. Hence, under basal conditions and with the observed range of values the percentage of ferritin was taken as a measure of the proportion of ferritin in total non hemin iron.

PATHOLOGICAL GROUPS VERSUS THE BASAL GROUP — In order to examine the relevance of some statements in the

[1] The subjects of groups *(v)* and *(vi)* are included in the groups *(ii)* and *(iv)*

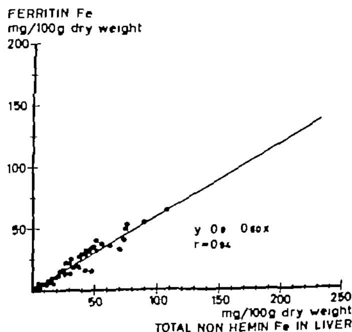


Fig. 14 Relation of ferritin iron to total non-hemin iron in the liver of the basal group. (n = 55 x = 20 s^2_y 109.3 ; s_y = 10.45)

rhosis group did not show any significant deviations from the basals. Nor was there any significant difference for a group with relatively high total non-hemin iron values and consisting of the more severe cases of infectious or toxic disease (the group Misc. $x > 100$ mg non-hemin iron /100 g dry wt.)

As regards the *bone marrow* the series were too small for analysis in a similar manner Since the above analysis did not disclose large deviations between the groups in the case of liver a rough ana lysis of the whole heterogeneous bone marrow material was made (Fig. 16) The relation of ferritin iron to total non-hemin iron in marrow expressed by the regression equation $\bar{y} = 3.3 + 0.60\,x$ did not differ essentially from that of the liver

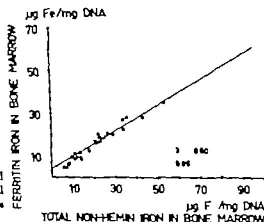


Fig 16. Relation of ferritin iron to total non-hemin iron in bone marrow The material comprised 44 subjects (basals and various pathologic states)

Discussion

Animal studies on the quantitative relationship between ferritin iron (water soluble) and hemosiderin iron (water-insoluble) in the liver at different states of iron load have shown that under normal conditions the greater part of the storage iron is present as ferritin iron When the non-hemin iron in the li er of the rabbit has reached a concentration of 300—400 mg/100 g, wet weight, any further increase in total non-hemin iron is associated almost exclusively with an increase in hemosiderin iron [116, 149 151 152]

It has been reported on the basis of experiments on the rat that after administration of diphtheria toxin in sterile inflammation and in hepatic damage caused by carbon tetrachloride the ferritin content falls while the hemosiderin fraction increases [155 157] According to Heilmeyer in infectious and toxic and liver disease the synthesis of ferritin is disturbed and the hemosiderin content may increase even though the total iron content may be unchanged. Thus, in inflammatory conditions an increase in stainable iron, as revealed by histo-

TABLE 24 *Relation of ferritin iron to total non-hemin iron in the liver for pathological groups compared with basal subjects.*

Group		n	Total non-hemin iron (mg 100 g dry wt) $\bar{x}$	Ferritin iron (mg 100 g dry wt) $\bar{y}_{obs}$	$\bar{y}_{calc}$	Mean difference $y_{obs}-y_{calc}$ $\bar{d}$	$s_{\bar{d}}$	Test of the difference $\|t\|$	P_t
Inf. I	Mild infection. No anemia. No recent hemorrhage	13	57 6	38.3	34 6	+ 3.8	1 7	2.2	< 0.05
Inf II.	Moderate infectious or toxic disease. No anemia. No recent hemorrhage	8	80.6	41 9	48.4	− 6.5	5 9	1 1	> 0.10
Inf A.	Anemia of infection No hemorrhage	16	73 8	45 0	44.3	+ 0.7	2.8	<1	> 0.10
Misc.	Infection or toxic state with total non-hemin iron $x >$ 100 mg 100 g dry wt	7	159 1	84 4	95.5	− 11.1	8..	1.35	> 0.10
Cirrh.	Cirrhosis of the liver No hemorrhage	4	34 8	24 4	21.8	+ 3.6	1.5	2.4	> 0.05 < 0.10
Recent hemorrhage.	No infection	12	27 6	11 8	16.5	− 4 7	1 7	2.77	< 0.05

In recent hemorrhage all but one of the cases had lower values than those expected from the basal equation. The mean observed percentage of ferritin in total non hemin iron in this group was 42% ± 5 1 The deviations of the individual values from the mean for the basal group however varied widely When one case (no 78) in which the ferritin value was only 10% of the expected one was excluded from the series, the ratio of ferritin iron to total non hemin iron for the remaining 11 subjects of the group was still significantly lower than for the basals ($\bar{d} = -4.3$ $s_{\bar{d}} = \pm 1\ 8$ $t = 2.39$) The equation of the linear regression for the group of recent hemorrhage was $\bar{y} = -0\ 81 + 0\ 46\ x$ ($r = 0\ 93$ $P < 0.001$)

For the group with mild infectious disease and no anemia the mean ferritin value was significantly but not considerably higher than for the basals. The equation of the linear regression in this group was $\bar{y} = 2\ 0 + 0\ 63\ x$ The mean percentage of ferritin iron was 67% ± 2.7 The difference from the 60% for the basal group was thus small

The other groups of infectious or toxic disease and the small liver cir

rhosus group did not show any significant deviations from the basals. Nor was there any significant difference for a group with relatively high total non-hemin iron values and consisting of the more severe cases of infectious or toxic disease (the group Misc. x > 100 mg non-hemin iron /100 g dry wt.).

As regards the *bone marrow* the series were too small for analysis in a similar manner Since the above analysis did not disclose large deviations between the groups in the case of liver a rough analysis of the whole heterogeneous bone marrow material was made (Fig. 16) The relation of ferritin iron to total non-hemin iron in marrow expressed by the regression equation $\bar{y} = 3.3 + 0.60\,x$ did not differ essentially from that of the liver

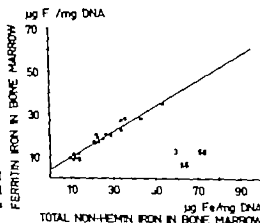


Fig. 16. Relation of ferritin iron to total non-hemin iron in bone marrow The material comprised 44 subjects (basals and various pathologic states).

Discussion

Animal studies on the quantitative relationship between ferritin iron (water-soluble) and hemosiderin iron (water-insoluble) in the liver at different states of iron load have shown that under normal conditions the greater part of the storage iron is present as ferritin iron. When the non-hemin iron in the liver of the rabbit has reached a concentration of 300—400 mg/100 g. wet weight, any further increase in total non-hemin iron is associated almost exclusively with an increase in hemosiderin iron [116 149 151 152]

It has been reported on the basis of experiments on the rat that after administration of diphtheria toxin in sterile inflammation and in hepatic damage caused by carbon tetrachloride the ferritin content falls while the hemosiderin fraction increases [155 15] According to Heilmeyer in infectious and toxic and liver disease the synthesis of ferritin is disturbed and the hemosiderin content may increase even though the total iron content may be unchanged. Thus, in inflammatory conditions an increase in stainable iron, as revealed by histo-

logic examination would not always imply an increase in total *non hemin* iron Recent experimental work indicates that hepatic damage induced by ethionine and N—2 fluorenylacetamide *in the rat* enchances the hepatocellular uptake of dietary *and colloidal iron* [159 160] It is, however questionable whether these results of animal experiments are applicable to liver disease in man.

The present study has shown that under basal conditions within a relatively wide range of physiologic iron load ferritin iron is on an average 60% of total non hemin iron with a normal *range of 24 to 96% The pathological* groups of infectious or toxic disease and even those of infectious disease that had a fairly high iron load displayed no difference from the basals as regards the *proportion of ferritin to total non hemin* iron For all but one of the pathological cases (no 61) the total non hemin iron concentration fell within the observed range for the basal subjects.

*Wohler et al [158 159] who deter*mined ferritin and hemosiderin iron by the method of Kelderling *et al* [10] in autopsy specimens from cases of different diseases, concluded that the ferritin concentration was greatly diminished in the liver or spleen in infectious or toxic and *liver diseases*, although hemosiderin iron could be actually increased However a number of the conclusions reached by these authors are not tenable since their comparisons were made using *only one control subject*. The consistently lower individual values for ferritin iron than hemosiderin iron *reported by* these authors are at variance with the present results the discrepancy may be due to the difference between the methods used.

The *present* findings are in close *agreement* with those *of* Morgan and Walters [79] who used comparable chemical methods for determining ferritin and hemosiderin iron On an autopsy material these authors found that the most important factor governing the distribution of iron between ferritin and hemosiderin was the total storage iron concentration With less than 50 mg/100 g wet weight, of liver tissue more iron *was stored as ferritin, whereas with* values above 100 mg/100 g more was stored as hemosiderin. In infectious, toxic and malignant disease no significant alteration was found in the *proportion of ferritin to hemosiderin iron* in liver tissue. For the spleen only in malignant disease was a small but significant alteration observed. However the mean concentration of total non hemin *iron in this group* was about three times that for the controls.

The fact that in the *present series* only the group of subjects with recent hemorrhage had considerable lower ferritin values is compatible with the current view that ferritin iron is more *readily* available than hemosiderin This is in accordance with Heilmeyer s results obtained on experimental animals [157] Morgan, however did not observe any change *in the proportion of* the storage iron fractions in the rat after hemorrhage [161]

Correlation between Concentrations of Non-Hemin Iron in Marrow and Liver

The bone marrow and the liver are the chief iron storage organs and contain about two-thirds of the total depot iron in the body [50] Since the marrow is the most easily accessible site for examination in hematological work it is of value to know whether there is any correlation between these two iron storage compartments. Such a comparison has been made on autopsy material by Hallgren [50] and, more recently by Gale *et al* [58] The subjects providing the material for these studies had died of various infectious, toxic or malignant diseases with, or without previous loss of blood, and it is possible that the distribution of storage iron between the two organs was influenced by these conditions. In the present study the concentrations of non-hemin iron in marrow aspirates and liver biopsy specimens were compared in basal subjects and groups of various diseases.

Material

Total non-hemin iron was determined simultaneously in bone marrow aspirates and liver biopsy specimens from 72 subjects. Of these, 35 were basals· that is, they had not had anemia or infection, nor a history of hemorrhage, at least during the year before study

The pathological groups consisted of (*a*) 9 cases of mild infection with no anemia or history of hemorrhage· (*b*) 7 cases of infectious or toxic anemia with no history of hemorrhage; (*c*) 6 cases of extra and intrahepatic cholestasis, 3 of whom had histological changes in the liver and fairly pronounced bilirubinemia (nos. 66 67 132) (*d*) 4 subjects with a history of recent hemorrhage and no concomitant infection. The remaining subjects were heterogeneous and too few to form suitable groups for comparison.

Results

In the basal group there was a highly significant correlation between the non-hemin iron concentration in the two organs over a relatively wide range of non-hemin iron concentration in liver tissue (Fig. 17). However the scatter of the individual values was appreciable.

The pathological groups were compared with the basal subjects as outlined above (p. 75) The non-hemin iron values for marrow were calculated (y_{calc}) by inserting the individual non-hemin iron concentrations for the liver into the equation for the basal subjects $y = 8.38 + 0.11 x$. From the differences between the calculated (y_{calc}) and observed (y_{obs}) concentrations of marrow iron

the mean difference ($\bar{d}$) for the group and its standard error ($s_{\bar{d}}$) were determined and the significance of the mean deviation from the basal line was examined by the t test

For the groups of (a) mild infection without anemia and (b) the anemia group the mean non hemin iron concentration in the bone marrow as related to the liver values was slightly higher

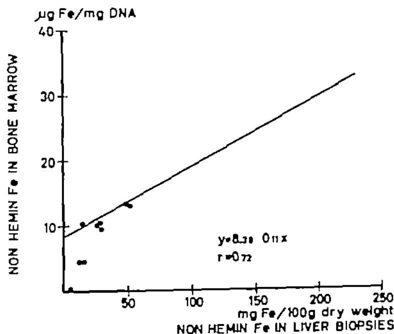


Fig. 17 Relation between total non-hemin iron concentrations in marrow aspirates and liver biopsy specimens, for the basal group (35 subjects) (t=5 96 P<0.001 s_y =5.3)

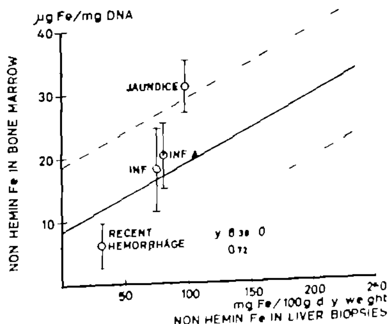


Fig 18 Relation between total non-hemin iron concentrations in marrow asp rates and li er biopsy specimens for pathological groups, compared with the regression line for the basal group. The figure ncludes the lines regression and th 95 per cent population limits for the basal group. The circles with th dot denote the me d f ference between the observed non hemin iron oncentrations in the m rrow and those cal culated from the basal equa tion. The ertical lines denote ± twice the standard error of the mean deviation. See lso Table 25

Table 25. *Relation of total non-hemin iron concentration in marrow aspirates and liver biopsy specimens in pathological groups as compared with the linear regression equation $y = 8.38 + 0.11\,x$ for the basal group. See Fig. 18*

Group		Total non-hemin iron: Liver (mg/100 g dry wt) $\bar{x}$	Bone marrow (mg/100 g dry wt) y_{obs}	y_{calc}	Mean difference $y_{obs} - y_{calc}$: $\bar{d}$	$\sigma_{\bar{d}}$	Test of the difference: $\lvert t \rvert$	P
Inf. Mild infection. No anemia or history of hemorrhage	9	74.6	18.2	16.6	+ 1.5	3.4	1.0	> 0.10
Inf. A. Anemia of infection or toxicity. No hemorrhage	7	78.9	20.4	16.7	+ 3.3	2.6	1.26	> 0.10
Jaundice. With and without hepatic damage	6	95.7	31.3	18.9	+ 12.4	4.15	2.99	< 0.05
Recent hemorrhage	4	31.1	6.2	11.8	− 5.6	1.8	3.1	> 0.05 < 0.10

but not significantly different from the mean for the basal group (Fig. 18, Table 25). For the jaundice group, however, the observed marrow values were significantly higher than for the basal subjects. The deviation of the mean from the regression line of the basals exceeded the 95% population limits of the curve (Fig. 18). However, the jaundice group was fairly small and the only subjects for whom there were pronounced deviations from the basal line were the 3 subjects with histological evidence of liver damage: nos. 66, 67, 132.

For the small group of recent hemorrhage the non-hemin iron concentration in the marrow was lower but not significantly different from the basals.

Discussion

It has been shown in animal experiments that in iron deficiency induced either by dietary restriction of iron in the growing animal [50] or by graded hemorrhage [161] the iron stores are reduced proportionately in the three storage organs — the liver, spleen and bone marrow. The storage iron of the carcass is less easily mobilized than that in these organs [50]. The values for storage iron in marrow and liver in cases of iron deficiency anemia of the present investigation, although not examined simultaneously in the same subject, suggest that there is a close correlation between the two organs also in man (Figs. 10 and 11). The presence of a close correlation in states of iron deficiency does not, however, imply that there is a similar correlation between the two organs in non-anemic normal subjects or other pathologic conditions than iron deficiency anemia.

There are anatomical differences between the storage of iron in liver and bone marrow In the liver the iron is stored mainly in the large parenchymal cell the histiocytic Kupffer cells contain visible iron only in the case of increased iron stores, in hemolysis or after injection of colloidal iron. In the bone marrow however as in the spleen storage iron is chiefly found in the reticulum cell The amount of non hemin iron seen in the red-cell precursors is small

In infectious or toxic diseases, which are often accompanied by different grades of hemolysis [129 134] there may be some shift of storage iron to the histiocytic iron storage cells in the marrow and spleen In fact, the only convincing increase in storage iron in post mortem studies of infectious or toxic disease has been found in the spleen [147 79] bone marrow was not examined Since the spleen is a small organ a moderate increase in storage iron would result in a significant increase in concentration whereas in marrow the increase might be so small as to be overlooked.

Electron microscopic studies by Bessis and co-workers [41 162] suggest that ferritin molecules from a reticulum "nurse" cell may be incorporated directly into the developing erythroblasts by a mechanism they call ropheocytosis According to this theory at least part of the iron for the formation of hemoglobin would come directly from the reticulo-endothelial iron stores in the bone marrow It is therefore possible that in cases of hemorrhage too slight to give rise to a state of iron deficiency the storage iron compartment of the marrow might be reduced to a greater extent than that of the liver How far quantitative conclusions can be drawn from the electron microscopic observations is questionable, since ferrokinetic studies indicate that most of the iron for hemoglobin formation is derived from plasma-bound iron

The present correlation studies on basal subjects disclosed a highly significant correlation between the iron stores in the liver and bone marrow however the correlation was far from absolute. The coefficient r of 0.72 accounts for only 52% of the variation Thus, though a low non hemin iron value in the marrow of a non anemic individual will *per se* indicate a fall in the iron stores we cannot with sufficient statistical confidence conclude that his stores are diminished to a comparable extent also in the liver It must be stressed that although a closer correlation at least for lower values, could probably be obtained by including subjects with established iron deficiency anemia, this is not the case for the normal individual.

Comparison of the pathological groups with the basal curve disclosed a slight but not significant increase in the mean bone marrow value in infectious or toxic disease. The jaundice group however had a significantly higher marrow value than that calculated from the equation for the basal subjects. For 3 out of the 6 subjects in the jaundice group the values were extremely high and there were histological signs of liver damage Although the group is too small

for generalizations, the findings are suggestive, at least for cases with active liver disease. Hepatocellular disease is often combined with hemolysis and, moreover storage iron may be released into the plasma from damaged liver cells [163]. This might cause a shift of iron into the marrow compartment.

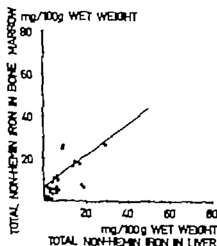


Fig. 19 Correlation of total non-hemin iron in marrow and liver with wet weight as reference examined by Hallgren on 41 specimens obtained at autopsy from subjects dying of various diseases. ($y = 6.3 + 0.73x$; $r = 0.56$; $t = 4.17$ $P < 0.001$; $= \pm 6.36$ calculated from Hallgren values [50]. (Courtesy of the author.)

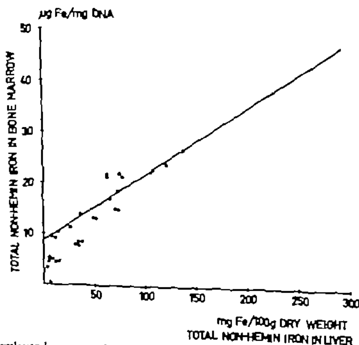


Fig. 20. Correlation between total non-hemin iron in marrow aspirates and liver biopsy specimens the present total unselected material of 72 subjects with DNA as reference for determinations in marrow (8.93 + 0.13 ; $r = 0.68$; $= 6.39$; $P < 0.001$; $s_y = \pm 7.7$).

It was considered of interest to compare the results of the present study in which DNA was used as a reference for determinations in marrow with the results of Hallgren [50] who determined non hemin iron in liver and marrow from ribs obtained at autopsy using wet weight as a reference. Fig 19 shows the scatter and linear regression line calculated from his 41 values, and Fig 20 the regression for the present unselected material comprising 72 subjects There is a similarity between the scatter of the values. In a recent study Gale *et al* [58] examined the above relations over a wide range of non-hemin iron concentrations, using *specimens obtained at autopsy* and found a closer correlation ($r = 0.88$) Their material included a large number of Bantu subjects with hemosiderosis. The graph presented by these authors indicates that the scatter would have been greater if the calculations had been based only on the lower concentrations usually encountered in the white population

TIBC as a Function of Non-Hemin Iron in Liver and Bone Marrow

It is established that the TIBC level is low in the newborn and rises gradually throughout infancy to levels above those found in the adult [88, 164]. In iron deficiency anemia, later pregnancy and acute hepatitis the TIBC is high [88, 165—168]. A depression of the TIBC concentration, usually accompanied by a subnormal serum iron level, is seen in all pathological conditions involving disturbance of the protein metabolism through synthesis or losses [169, 103]. Thus, a low TIBC is found in acute and chronic infection, malignancy, nephrosis, severe liver disease and kwashiorkor [88, 170—173]. In hemolytic and megaloblastic anemia, refractory anemias with iron overload and in hemochromatosis the TIBC is low and its percentage saturation with iron high [88, 174].

It has been suggested by Laurell and others [88, 103] that there is a negative correlation between the iron stores and the TIBC, and support for this is found in the usual association of enlarged or well filled iron stores and subnormal TIBC in the above diseases, and in the observations on non-anemic blood donors in whom the TIBC seemed to be increased [175]. The depression of the TIBC in the above diseases may however be due entirely to the disease itself and the high TIBC in blood donors may be due to phlebotomies, temporary anemia, increased erythropoiesis and altered ferrokinetics. Moreover, with regard to blood donors conflicting results have been reported [87]. In another study it has been found that TIBC was a reliable index of iron deficiency only when the hemoglobin level was below 9.0 g/100 ml [119].

The present investigation was designed to study directly the pattern of the relationship between the TIBC and serum iron, on the one hand, and the non-hemin iron concentrations in the liver and bone marrow on the other. A negative correlation between the TIBC level and the iron stores was assumed as a working hypothesis in non-anemic, healthy subjects without hemorrhage. As it was anticipated that the largest changes in the TIBC level would be associated with pronounced diminution of the storage iron concentration, the problem was examined on a group part of which was assumed to represent the normal range of non-hemin iron concentration, the other part was expected to have excessively low iron stores but without a history of hemorrhage and still having a normal hemoglobin level. The latter group was com-

posed of patients that had had a Polya gastrectomy

The relation between the TIBC and storage iron was compared for basal and pathological subjects

Material

Group A provided liver biopsy specimens for comparison between the *non hemin iron concentration* and the TIBC and serum iron levels group B *provided bone marrow aspirates for the* same analyses. Each of these groups was divided into 'basal *and non basal* subjects. The basal subjects had a normal hemoglobin concentration ($\geq$ 13.5 *g per* 100 ml for men and $\geq$ 11.5 g for women) there were *no signs of infection and no* history of nonphysiological blood loss during the year prior to study

Group A — Forty four basal subjects *(28* men, *8 post menopausal and 8* men struating women) Forty three non basal *subjects. Their subdivision into patho*logical groups suitable for comparison *with the basals is shown in* Table 26

Group B — Eighty-eight basal subjects (70 males, 7 non menstruating and 11 menstruating women) Forty six of the males had *had a Polya* gastrectomy Eighty five non basal subjects the composition *as shown in Table 27*

On 25 of the 88 basal subjects of group B *simultaneous non hemin iron* determinations were performed in liver *tissue and they are therefore included* among the basal subjects of group A. The pathological groups, too contain subjects included in groups A and B The rest of the examined non basal subjects who are listed in the *Appendix* but not included in tables 26 and 27 were heterogeneous or too few to be suitable for analysis.

Results

BASAL GROUPS. — Linear regression analysis of the relation between the TIBC level and the non hemin iron concentration in liver (group A) and bone marrow (group B) disclosed a highly significant negative correlation in both groups. For group A the equation of the linear regression was $\bar{y} = 349.5 - 0.603\,x$ ($r = -0.46$ $t = 3.34$ $P < 0.001$) For group B the equation was $\bar{y}_x = 374.4 - 3.61\,x$ ($r = -0.49$ $t = 5.2$ $P < 0.001$)

Although a negative correlation between TIBC and non hemin iron concentration in both liver and bone marrow *was established it was apparent* from the scatter diagrams that the linear regression line did not properly fit the pattern of this dependence. When groups A and B were divided into two equal parts one with low and the other with high non hemin iron concentrations it was found that the slope of the regression line was greater for the former than the latter for which it was small and not significant. *This difference* was more pronounced in group A, in which there *was a relatively* wide range of non hemin iron concentrations in the liver tissue

Trials with other mathematical models showed that the relationship for basal subjects was best fitted by the second degree parabola $\bar{y} = a + bx + cx^2$ This equation was used as a model for the observations up to the value for the

non-hemin iron concentration x for which the function is a minimum. The minimum TIBC, $\bar{y}_{min}$ is $a - b^2/4c$ For values of x (non-hemin iron concentrations) greater than x $\bar{y}$ is constant and equal to $\bar{y}_{min}$

For the liver the range of the non-hemin iron values was 4.0 to 227.0 mg Fe/100 g, dry weight, mean 52.3 (Fig 21). The lowest mean TIBC value (y_{min}) of 245 μg/100 ml corresponded to a non-hemin iron concentration (x') of 160 mg Fe/100 g, dry weight. The correlation coefficient was —0.72, against —0.46 according to the equation of the linear regression. The difference between these coefficients is, however not significant.

The relation of TIBC to non-hemin iron concentration in bone marrow for

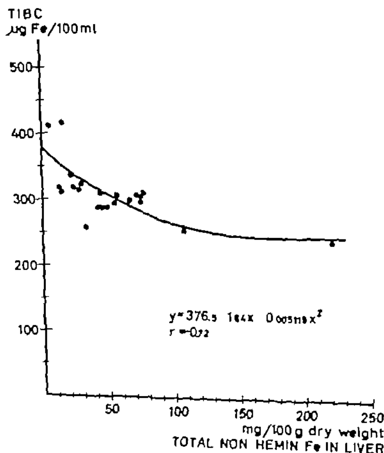


Fig. 21 Relation between the total iron-binding capacity and concentration of total non-hemin iron in liver biopsy specimens for the basal group (44 subjects). (= —6.8 ; $P<0.001$; = ±35.2).

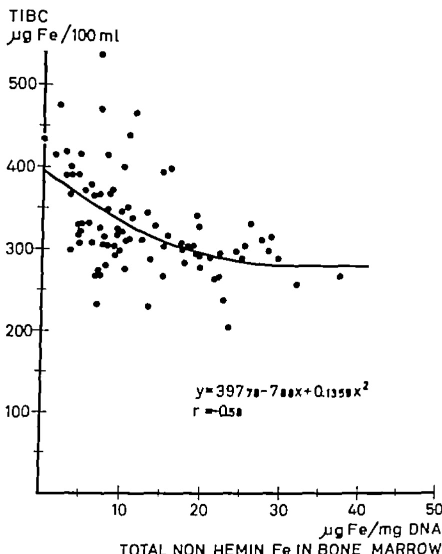


Fig 22 Relation between the total iron-binding capacity and concentration of total non-hemin iron in bone marrow asp rates for the basal group (88 subjects) ($t = -6.7$ $P < 0.001$ s_y $= \pm 46$)

basal subjects is shown in Fig 22 For the marrow the range of non hemin iron values was 0 0 to 36 9 μg Fe/mg DNA mean 12.3 From the equation the lowest mean TIBC, y_{min} of 284 μg Fe/100 ml corresponded to a non hemin iron concentration x of 29 0 μg Fe/mg DNA. The correlation coefficient was —0 58 against —0 49 according to the equation of the linear regression It may be noted that the lowest TIBC value (y_{min}) in this equation was 284 against 245 in the equation for group A. This difference in the value of y_m obtained for groups A and B is due to the different values and ranges for non-hemin iron concentrations (values of x) represented by these two curves. In group A a wide range of values of x is represented, and the mean $\bar{x}$ of 52.3 mg of

non-hemin iron per 100 g dry weight is considerably higher than the corresponding mean for group B of 12.3 μg Fe/mg DNA. This dependence of y_{mean} on the ranges of the values of x represented by the curve must be kept in mind when other groups of subjects are to be compared with the basals.

The pathological groups were compared with the respective curves of the basal subjects in groups A and B according to the principles outlined above (p. 75)

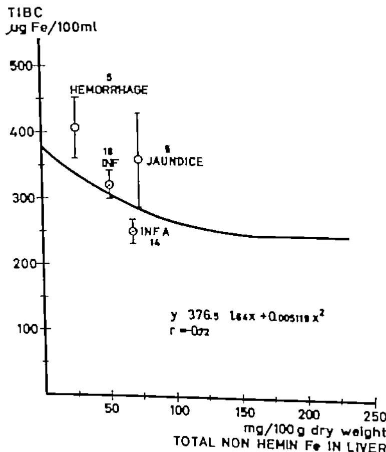


Fig. 23 Relation between the total iron-binding capacity and non-hemin iron concentration in liver biopsy specimens for pathological groups, compared with the regression line for the basal group. The circles with the dot denote the mean deviation of the observed TIBC values from the basal curve. This deviation is the mean difference between the observed TIBC and the values calculated from the basal equation. The vertical lines denote ± twice the standard error of the mean deviation. The numbers give the size of the group. For abbreviations and description of the groups see Table 26.

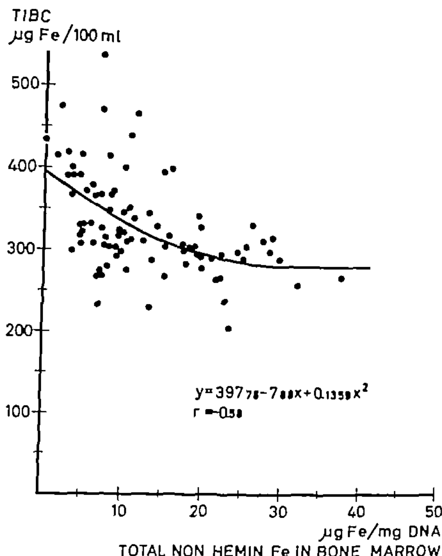


Fig 22. Relation between the total iron-binding capacity and concentration of total non-hemin iron in bone marrow aspirates fo the basal group (88 subjects). ($t = -6.7$ $P < 0.001$ $s_{y.x} = \pm 46$).

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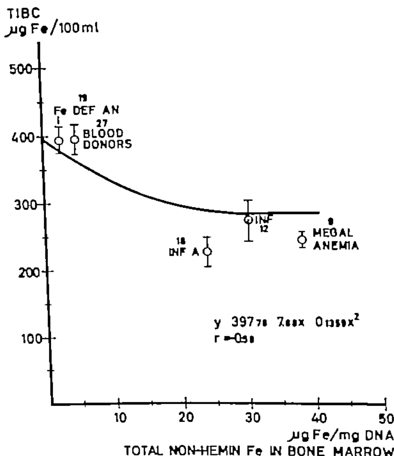


Fig. 24 Relation between the total iron binding capacity and non-hemin iron concentration in marrow aspirates for pathological groups compared with the regression line for the basal group. For explanation see legend to Fig. 23. For abbreviations and description of the groups see Table 27

a significantly lower mean TIBC than the calculated. For all but one of the subjects of this group the TIBC values were lower than those calculated from the equation of the basal curve. Even if the 3 subjects with a non-hemin iron concentration higher than the range for the basal group (nos. 265 266 271) are excluded the difference would still be significant ($\bar{d}$ −71 9 $s_{\bar{d}}$ = 12.8; t = −5.6 $P < 0.001$). The group of infection without anemia (mild infection) did not differ from the basals.

The megaloblastic anemia group had a significantly lower TIBC level than that expected from the equation. For each subject in the group the value was lower than calculated. If the 5 of the 9 subjects with non hemin iron concentrations above the range of the basal

TABLE 26 *Relation of TIBC and tot l non-hemin on in liver in pathol gical groups as compared with the semipa abolic regression equation* $\bar{y} = 376.5 - 1.64\,x + 0.005119\,x^2$ *for the basal subjects. For each subject the TIBC value was calculated by inserting its liver value (x) in the above equation. The mean difference* ($\bar{d}$) *between the observed and calculated values fo each group was exam ned by the t test See Fig 23*

		Total non-hemin Iron in liver (mg/100 g dry wt) $\bar{x}$	TIBC (g/100 ml) $\bar{y}_{obs}$	$\bar{y}_{calc}$	Mean difference $y_{obs} - y_{calc}$ $\bar{d}$	$s_{\bar{d}}$	Test of the difference $\|t\|$	P
Inf Infection. No anemia	16	50 5	318	305	+ 12.7	10.6	1..	> 0 10
Inf. A. Anemia of infection	14	67.2	267	306	− 39.5	9 1	4.34	< 0.001
Recent hemorrhage	5	25 1	411	342	+ 69.2	22.6	3.06	0.05
Jaundice	5	72.3	364	292	+ 72.0	35.5	2.03	0 10 > P > 0.05

Cases of liver and biliary disease with jaundice were eliminated from the groups of infectious diseases because their TIBC level may be elevated [88 168] This increase concomitant with the rise in the serum iron level has been ascribed to the presence of ferritin in plasma released from the damaged liver cells [163 103]

GROUP A LIVER PATHOLOGIC SUBJECTS (Fig. 23 Table 26) — All non-basal subjects but one (case 61) had non hemin iron concentrations in liver within the range of the basal group

The observed mean TIBC for the group of anemia of infection was significantly lower than that calculated by insertion of their non hemin iron concentrations into the regression equation for the basal subjects. For all but one of the cases of this group the TIBC values were lower than those calculated. Exclusion of case 61 who had extremely high non hemin iron values, would not alter the results. The group of mild infections without anemia did not differ from the basal subjects. The mean TIBC for the small group of 5 subjects with obstructive jaundice was higher than expected from the regression line of the basals but the difference was not significant.

For a group of 5 subjects with a history of recent hemorrhage one of whom (case 95) had iron deficiency anemia, the TIBC was significantly higher than calculated from the equation of the basals.

GROUP B BONE MARROW PATHOLOGIC SUBJECTS (Fig 24 Table 27) —The TIBC was slightly but not significantly higher for the group of iron deficiency anemia than for the basal group For the blood donors, however it was significantly higher than calculated from the regression equation of the basal group The group of infectious or toxic anemia had

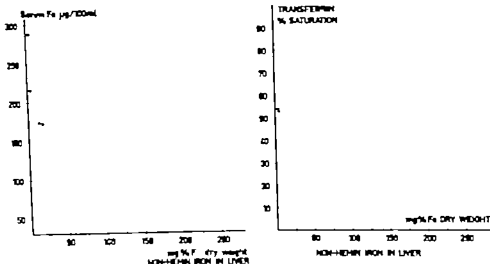


Fig. 25 A and B. The relation of serum iron and of the transferrin saturation to non-hemin iron concentration in liver biopsy specimens; basal group (44 subjects).

Serum F µg/100ml
300
250
200
150
100
50
10 20 30 40
µg F /mg DNA IN BONE MARROW

TRANSFERRIN
% SATURATION
90
80
70
60
50
40
30
20
10
10 20 30 40
µg Fe/mg DNA
NON-HEMIN IRON IN BONE MARROW

Fig. 26 A and B. Relation of serum iron and transferrin saturation to non-hemin iron concentration in marrow aspirates; basal group ([illegible] subjects).

TABLE 27 *Relation of TIBC and total non-hemin iron concentration in marrow in pathological groups compared with the regression equation $\bar{y} = 397.8 - 7.88\,x + 0.1359\,x^2$ for the basal group. The deviation of the pathological groups from the basal curve was calculated in the same way as described in legend to Table 26. The significances of these deviations were examined by the t-test*

		Total non-hemin iron in marrow (μg Fe/mg DNA)	TIBC (μg/100 ml)		Mean difference $y_{obs} - y_{calc}$		Test of the difference	
	n	$\bar{x}$	$\bar{y}_{obs}$	$\bar{y}_{calc}$	$\bar{d}$	$s_{\bar{d}}$	$\lvert t \rvert$	P
Fe def an., Iron deficiency anemia	19	4.6	397	379	+17.6	9.7	1.8	0.10 > P > 0.05
Blood donors	27	4.9	395	363	+32.1	10.9	2.94	< 0.01
Inf., Mild infection, No anemia	12	29.1	300	310	−9.8	15.5	< 1	> 0.10
Inf. A., Anemia of infection	18	23.3	246	309	−62.7	10.7	5.86	< 0.001
Megaloblastic anemia	9	38.0	243	285	−42.0	5.7	7.37	< 0.001

group are excluded the deviation from the basal curve is still significant ($\bar{d} = 49.3$, $s_{\bar{d}} = 7.7$, $t = -6.4$, $P < 0.05$).

Cases of jaundice in group B were few and the variations too great for inclusion in the statistical analysis. With the exception of 2 cases of severe biliary cirrhosis who had very low TIBC values (nos. 66, 67) there was, however, a tendency for a high TIBC.

Analysis of the relations between the TIBC level and the non-hemin iron concentration in the liver by the straight regression line in the 13 cases of anemia of infection showed a significant and negative correlation ($y = 284.9 - 0.27\,x$, $r = -0.61$, $t = -2.59$, $P < 0.05$). There was a negative correlation between TIBC and non-hemin iron concentration in the bone marrow of 17 subjects with anemia of infection, but it was not significant.

Relation of non-hemin iron to serum iron and transferrin saturation[1]

In liver and marrow of the basal subjects there was no significant correlation between serum iron and non-hemin iron concentrations (Figs. 25a, 26a).

Since there was a significant negative correlation between TIBC level and non-hemin iron concentration, a negative correlation between the percentage transferrin saturation and non-hemin iron would be expected if the serum iron decreased or remained unchanged with increasing TIBC. There was, however, no evidence of such a relationship (Figs. 25b and 26b) ($r = -0.04$). This implies that at least in some of the subjects the serum iron level rose with TIBC concentration to maintain saturation.

[1] Ratio of serum iron to total iron binding capacity times 100.

TABLE 28. *Total non-hemin iron concentration in marrow and the TIBC level in two groups of post-gastrectomized subjects before and after 3 months of oral iron administration. Group A with initial non-hemin iron concentrations below 10.5 μg Fe/mg DNA. Group B with concentrations above 10.5 μg. t-test of the means from before and after iron administration.*

		Total non hemin iron (μg Fe/mg DNA)			Test of difference		TIBC (μg/100 ml)			Test of difference
		Before $\bar{x}$	After $\bar{x}_2$	$\bar{x}_2-\bar{x}$ $\bar{d}$	$\|t\|$	P	Before $\bar{y}$	After $\bar{y}$	y_1-y $\bar{d}$	$\|t\|$
μg Fe/mg DNA	15	7.0	11.4	$+4.33 \pm 1.3$	3.33	< 0.001	355	320	-34.7 ± 13.1	2.65
μg Fe/mg DNA	7	20.8	23.9	$+2.8 \pm 1.7$	1.68	> 0.10	321	305	-16.4 ± 11.5	1.4

In the second group with higher initial non-hemin iron values (> 10.5) there were no significant changes in non-hemin iron concentration or TIBC.

Changes in TIBC following phlebotomy

This study was performed on two groups of blood donors, one consisting of 7 subjects who for 5 months prior to study had been given 50 mg of ferrous glycine sulfate solution twice a day and the other 3 subjects who had received 1600 mg of colloidal iron intravenously. The total mobilizable iron stores of these subjects were determined by the method of Haskins and co-workers [125] on 500 ml volumes of blood drawn weekly until retardation of hemoglobin regeneration was achieved. The oral iron group had about 300 mg and the group receiving colloidal iron about 1300 mg of mobilizable storage iron [176]. The TIBC of these subjects had been determined weekly prior to each phlebotomy.

The maximum increase in the TIBC in the group with low iron stores occurred after 5 phlebotomies and in the other group after 11 phlebotomies. Though the experimental conditions for the two groups were similar and the degree of anemia developed by phlebotomies was of the same degree, the TIBC did not reach its maximum in the group with filled iron stores until these had been exhausted (Fig. 27).

Discussion

In hematologically normal subjects there was a significant negative correlation between the total iron binding capacity of serum and the concentration of storage iron in liver and bone marrow. Pathological groups of subjects showed some deviations from the basals but the pattern was essentially the same, in that a high TIBC was associated with low storage iron concentration, and conversely. This negative relation between the above parameters was curvilinear, changes in TIBC level being greater when the storage iron concentrations were low than when they were well filled. The results indicate that the iron stores

On dividing the 44 subjects of the basal liver group into two equal groups, one with non hemin iron up to 42 4 mg/100 g dry weight and the other above this value, it was found that the mean serum iron level for the first group with low iron stores was 161 ±12.2 μg/100 ml with a standard deviation of ±57 4 whereas for the second group the mean was 140 ± 7.2 and a standard deviation of ± 34 Although the differences between the mean serum iron values and between the standard deviations of the serum iron values of these two groups were not statistically significant, the figures suggest a larger scatter with a higher mean serum iron for the subjects with low iron stores. The means for the percentage transferrin saturation for both groups were almost identical (48% ± 4 and 50% ± 2.7 respectively) The corresponding standard deviations were ± 18 7 and ± 12.7 Thus, although the difference between these standard deviations was not significant a tendency towards a greater dispersion of values for the same mean percentage saturation in the group with low iron stores was suggested.

A similar analysis was performed on the 88 basal subjects of group B (bone marrow) After division of these subjects into two equal groups, one with low and the other with high iron stores, similar means were obtained for serum iron (141 ± 6 6 and 142 ± 6 0 μg/100 ml) and the percentage transferrin saturation (42% ± 2 1 and 47 ± 2.2) In the case of bone marrow there was no appreciable difference between the standard deviations of the groups with low and high storage iron.

Additional studies

Further direct and indirect evidence of a negative correlation between iron binding capacity of serum and storage iron concentration in hematologically normal subjects was yielded by the following studies.

Non hemin iron and TIBC before and after iron administration

Non hemin iron in bone marrow aspirates and serum iron and TIBC level were determined in 22 subjects who had had a Polya gastrectomy many years previously All had normal hemoglobin values and there was no history of hemorrhage. After these determinations had been performed the subjects were given ferrous glycine sulfate solution in 2 daily doses of 50 mg Fe^{++} each for 3 months. The second determination of non hemin iron in bone marrow and of the TIBC level was performed 14 days after the last administration of iron Between the two sternal punctures the hemoglobin serum iron and TIBC levels were checked in all subjects at monthly intervals. The whole group was divided according to whether the non-hemin iron concentration in the bone marrow at the first examination was less or more than 10 5 μg Fe/mg DNA (15 and 7 subjects, resp) The differences between the non hemin iron and TIBC before and after iron administration were tested in both groups the results are summarized in Table 28 For the group of subjects with low non-hemin iron concentrations (≤ 10 5) there was a significant increase in the non hemin iron and a significant decrease in the TIBC after iron therapy

Table 28. Total non-hemin iron concentration in marrow and the TIBC level in two groups of post-gastrectomized subjects before and after 3 months of oral iron administration. Group A with initial non-hemin iron concentrations below 10.5 µg Fe/mg DNA. Group B with concentrations above 10.5 µg. t-test of the means from before and after iron administration.

		Total non-hemin iron (μg Fe/mg DNA) Before $\bar{x}$	After $\bar{x}_2$	$\bar{x}_2 - \bar{x}$ $\bar{d}$	Test of difference $\lvert t \rvert$	P	TIBC (μg/100 ml) Before $\bar{y}$	After $\bar{y}_2$	$\bar{y}_2 - \bar{y}$ $\bar{d}$	Test of differen[ce] $\lvert t \rvert$
g Fe/mg DNA	15	7.0	11.4	$+4.33 \pm 1.3$	3.33	< 0.001	355	320	$-34.7 - 13.1$	2.65
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Discussion

In hematologically normal subjects there was a significant negative correlation between the total iron binding capacity of serum and the concentration of storage iron in liver and bone marrow. Pathological groups of subjects showed some deviations from the basals but the pattern was essentially the same, in that a high TIBC was associated with low storage iron concentration, and conversely. This negative relation between the above parameters was curvilinear, changes in TIBC level being greater when the storage iron concentrations were low than when they were well filled. The results indicate that the iron stores

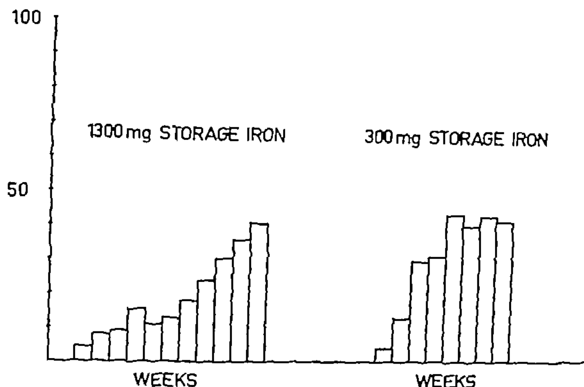


Fig. 27 The percentage increase of the total iron-binding capacity following weekly phlebotomies of 500 ml blood in a group with a mean with 1300 mg and another with 300 mg of storage iron. The former group consisted of 3 and the second of 7 subjects. The bars denote the mean percentage increase in TIBC one week after each phlebotomy from the basic mean for the group before the phlebotomies.

are the main regulator of the TIBC level in serum although other major factors such as disturbed protein metabolism may influence it independently This confirms Laurell's suggestions and is consistent with most clinical observations

Iron stores are relatively high in the newborn and then fall to very low values during the second year of life [105 106] Correspondingly the TIBC is low at birth, and rises gradually in infancy to levels above those found in adulthood [88 164]

In megaloblastic anemia in relapse the iron stores are augmented and the TIBC is lowered [88] After administration of B_{12} or folic acid there is a rapid fall in the serum iron levels the TIBC, however remains fairly constant during the first weeks of therapy but then when the iron stores have been mobilized or exhausted by the steadily increasing hemoglobin mass it assumes normal or high values [177 178] Besides the iron stores, there is probably some other factor that is responsible for the initial

low TIBC since, as this study has shown, the mean TIBC in megaloblastic anemia in relapse is significantly lower than that calculated from the equation for the basal subjects.

In iron deficiency anemia it has been observed that during supply of ferrous salts the TIBC decreases before the serum iron level and anemia have been corrected [178 179] This reduction in the TIBC was slow and occurred after the first 2—3 weeks of therapy [178] During iron deficiency anemia the absorption of iron is greatly enhanced and patients receiving about 300 mg of ferrous salt a day are likely to absorb iron in excess of the hemoglobin formation, so that after several weeks of therapy a depot of about 200—300 mg of iron may be built up which is enough to bring the TIBC level down towards normal It is possible that if therapy were discontinued and this fugitive depot drawn upon for hemoglobin formation the TIBC level would rise again The above observations in megaloblastic anemia and iron deficiency anemia during specific therapy also show that the TIBC varies independently of the serum iron concentration. The TIBC values found in pregnancy refractory and hemolytic anemias and in hemochromatosis support the assumption that the iron stores are the basic regulator of the transferrin level

It is not known in what way the iron stores m y influence the TIBC but the following three possibilities may be considered. () Storage iron may bind the transferrin molecules within or to the surfaces of the cells and produce a shift of the protein to the plasma when the iron stores become depleted. (*ii*) A decrease in the concentration of storage iron in the liver and reticulo-endothelial cells may accelerate production of the protein. (*iii*) Storage iron may influence the rate of degradation of the protein. The first hypothesis finds support in the observations of Mitchell and co-workers [180] that a large oral dose of an iron salt causes a rapid fall in the TIBC concentration in subjects with iron deficiency anemia. These authors advance the hypothesis that transferrin exists in two phases, one circulating and the other at cellular sites, and that iron transfer into cells is accomplished by sequestration of the iron-containing transferrin molecule to iron receptors on the surface of the cell. This hypothesis is supported by the *in vitro* studies of iron-transferrin uptake by reticulocytes [181] Laurell, however did not find any changes in the transferrin level after oral and intravenous administration of iron, to normals or to subjects with iron deficiency anemia or subacute infection [88, 169] Since the TIBC remained constant in various experiments in which rapid changes in the serum iron level were induced, Laurell concluded that iron is dissociated from the transferrin molecule before it leaves the circulation. He postulates that the transferrin clearance and plasma iron clearance are two independent processes. In a recent stud Awai and Brown [179] found no evidence that transferrin is rapidly withdrawn from plasma after administration of large doses of oral iron or intravenous infusions of ferrous sulfate to subjects

with iron deficiency anemia. These authors question whether the sequestration of iron laden transferrin on the surface of reticulocytes shown *in vitro* [181] occurs *in vivo* and consider that if it does it cannot be to a large extent. Their metabolic study on labelled human transferrin indicate that the decrease in the TIBC level encountered in the course of treatment for iron deficiency anemia is due to retardation of synthesis in the face of continued high rates of degradation. The solution of these problems must apparently await further investigation.

Since in the basal group the non hemin iron was not correlated to the serum iron or the percentage transferrin saturation and since there was a tendency towards a greater dispersion of the values of these two factors at low non-hemin iron concentrations, it was assumed that, at least in part of the sample, the serum iron must have increased with rising TIBC so as to maintain saturation. This would be consistent with Laurell's hypothesis that there is *in vivo* an equilibrium between iron ions and unsaturated and saturated transferrin [88]. However when no more iron is available and no increase in absorption from the gut is possible the serum iron concentration and the percentage transferrin saturation will decrease. This sequence of events is suggested by the pattern of the scatter of serum iron and percentage transferrin saturation in hematologically normal (basal) subjects with low iron stores (Fig. 25A, 25B) this pattern may be explained by supposing that the population with low iron stores is composed of two kinds of subjects — those who can and those who cannot increase the serum iron concentration and equilibrate the transferrin saturation. There would be a greater chance of the latter developing iron deficiency anemia. It is notable that in a study by Pirzio-Biroli and Finch [87] subjects who had been phlebotomized initially had low serum iron values, but after recovery from anemia the serum iron levels were higher than for subjects with loaded iron stores. Their TIBC was not recorded. This would seem to be consistent with the above reasoning that in non anemic subjects with low iron stores and high TIBC the serum iron concentration may increase provided that iron is available either in the still incompletely exhausted stores or at the sites of intestinal absorption.

It is established that the absorption of iron is in some measure dependent on the size of the iron stores [86, 87] and it has also been shown that even in normal non anemic subjects a decrease in the iron stores results in an increase in iron absorption [87]. However it is not known how the lowered iron stores of the non anemic individual induce the intestine to increase iron absorption. Nor is it known at which stage of contraction of the iron stores in the normal subject the augmentation in iron absorption is large enough to be discernible. Since, as found in this study there is an increase in the transferrin level as the iron stores are lowered in the normal subject, Laurell's hypothesis that the level of unsaturated transferrin may to some extent regulate iron absorption and mobilization from the stores

must be considered [88]. This theory has recently found support in experimental human studies by Hallberg and Sölvell [89] and in animal studies by Charley and co-workers [90]. However, other workers have shown in animal experiments that iron absorption continues even when the transferrin is saturated [91, 92]. Even so this does not rule out the possibility that the absorption increases when the level of unsaturated transferrin is high. It may be questioned, moreover, whether conclusions drawn from these experiments are applicable to physiological conditions in man.

If Laurell's hypothesis is accepted, it might be anticipated that the increase in absorption that occurs when the stores are low is effected through an increase in the TIBC. Figures 21 and 22 suggest that a discernible increase in absorption is to be expected at the left part of the x axis when the non-hemin iron concentration in the liver is below 40 mg/100 g. dry weight, and in the bone marrow below 12 μg/mg DNA. If the storage iron concentrations are higher than these, moderate changes in iron stores will probably be followed by only small, perhaps scarcely discernible, variations in iron absorption.

Histochemical Estimation of Iron Stores and the Early Diagnosis of Iron Deficiency Anemia

Introduction

For diagnosis of iron deficiency anemia the presence of hypochromia and microcytosis of the circulating red cells have been considered essential. However iron deficiency may retard erythropoiesis for a long time before these morphologic abnormalities become recognizable Furthermore, they may be found in other conditions in which the hemoglobin synthesis is depressed, including anemia or infection lead poisoning thalassemia and the hemoglobinopathies [182 183 95] By determining iron [184—187] and the total iron binding capacity in serum [88] iron deficiency can be detected at an early stage however these tests may be complicated by the presence of infectious and toxic diseases, and conditions involving disturbances of protein metabolism [88 187 188] Since the first consequence of a negative iron balance is a progressive decrease in the iron stores, a determination of their level provides a more direct means of demonstrating iron depletion. The chemical determination of non hemin iron in bone marrow described above is laborious and unsuitable for routine work. A practical method for assessing storage iron in marrow is that introduced by Rath and Finch [189] by which reticulo-endothelial hemosiderin is estimated semi-quantitatively in either unstained or stained smear preparations or histological sections It has frequently been reported that reticulo-endothelial hemosiderin is consistently absent in established chronic iron deficiency anemia but normal or increased in other anemias [118—120 122 123] However since chronic iron deficiency anemia implies empty iron stores by definition the consistent absence of hemosiderin in this condition does not necessarily prove that similar findings in other situations have the same significance [124] In spite of the absence of reticulo-endothelial hemosiderin, storage iron may still be present in the form of microscopically invisible ferritin

Another means of evaluating the state of the iron stores is the sideroblast count. This was introduced as a laboratory test by Douglas and Dacie [121] and Kaplan *et al* [190] who have shown that the sideroblasts are absent or greatly reduced in iron deficiency anemia. The diagnostic value of this test has been questioned, since sideroblasts may be absent even when the content of reticular hemosiderin is normal [119 191] A study of this discrepancy in different clinical conditions revealed that the iron pool

forming the sideroblasts is small and readily available and may therefore undergo rapid changes [120 124] Accordingly in conditions in which there is an accelerated formation of hemoglobin and the rate of iron mobilization from the stores not fast enough the sideroblasts may disappear even when the storage iron has not yet been consumed. After recent hemorrhage and after administration of B_{12} to patients with pernicious anemia in relapse a reduced sideroblast count and a normal amount of reticular iron were often found [124] On the other hand, during administration of oral iron for iron deficiency anemia, the supply of excess iron to the erythroblasts may result in a normal sideroblast count even though the iron stores are quite empty In these conditions the changes in the sideroblast count followed those in the serum iron levels. Another exception is active infectious or toxic disease, in which a low sideroblast count may be recorded in spite of ample iron stores. A count as low as that in iron deficiency anemia has been observed in about 10% of a series of cases of inflammatory or toxic disease associated with hypoferremia [124]

The present study is concerned with the relation of stainable reticulo-endothelial iron and of the sideroblast count to chemically determined non-hemin iron and to the transferrin and its iron.

Material and methods

The main material for the analysis consisted of the data presented in the tables in the *Appendix* An additional 34 subjects were used for the study of the relation between stainable reticular iron and chemically determined non-hemin iron in bone marrow on 5 others an analysis of the sideroblast relationships was made

The examination for a correlation between stainable reticular iron and chemically determined total non-hemin iron was performed on the material as a whole. However in the study of the relation between the percentage of sideroblasts and other parameters of iron metabolism due account was taken of the above clinical entities that might influence the sideroblast count [120 124] Accordingly the following groups were distinguished: (1) Basal subjects 66 men, 9 non-menstruating and 9 menstruating women. This group is defined as hematologically normal with no signs of infection and no history of hemorrhage, at least during the year before study Forty five of the male subjects had had a Polya gastrectomy They were all working and they attended the Out patients Department for a follow-up examination. (2) Chronic iron deficiency anemia 20 subjects. (3) Blood donors 25 male subjects. Five of them were slightly anemic (are not included in the tables of the *Appendix*). On all of them the assays were performed 5 or 6 weeks after the last blood donation. The characteristics of the group have been described above (p. 58). (4) Recent hemorrhage 11 cases. Six of them were anemic. (5) Infection with hypoferremia 13 cases. All had serum iron levels below 60 μg (range 21—53), and 10 were anemic, and their marrow smears

showed normal or increased reticular hemosiderin (6) Infection, with a serum iron level above 60 μg/100 ml 25 subjects. Fourteen had anemia and 4 of these had no visible reticular hemosiderin but normal total non hemin iron concentrations in the bone marrow For none of them was iron deficiency the cause of the anemia. (7) Other anemias 11 cases, 9 of whom had megaloblastic anemia.

The group of iron deficiency anemia and recent hemorrhage excepted none of the subjects in the other groups had a history of hemorrhage.

For staining and examining reticular hemosiderin and sideroblasts in smear preparations and in histological sections the technics previously described were used [120 192] Samples in which the amount of the material was deemed unrepresentative were discarded A grading scale of 0 to 4 + was used according to principles described elsewhere [192] In the case of smears 50 oil immersion fields were usually examined If hemosiderin was absent at least two slides were examined thoroughly Only intracellular hemosiderin was evaluated however if only traces of iron were found it was often difficult to decide whether or not the particles were situated within the cells as a rule one conventional smear preparation and one thick squash preparation were examined. Most of the smears were assayed independently by two examiners. The histological sections were evaluated at another laboratory For the calculation of the percentage of sideroblasts 100 erythroblasts were counted under oil immersion with a magnification of 1200 × [120]

The error of counting for the same examiner calculated from duplicate determinations made on 100 erythroblasts was, ± 5 6 corresponding to a coefficient of variation of 13 per cent The error of counting was suggested to be considerably higher when results from two examiners were compared. The differences were however least divergent when the sideroblast count was lower than 12 per cent.

Stainable reticulo-endothelial iron

Between the stainable reticular hemosiderin in marrow *smears* and the chemically determined total non-hemin iron studied in 242 subjects, there was a highly significant correlation (Fig 28 Table 29) For any histochemical group the mean of the chemical determination was significantly different from that of either of the adjacent groups. Although higher than the means for the iron deficiency anemia group (2 4 ± 0.4 μg Fe/mg DNA) and the blood donors (4 9 ± 0 4) the mean of the chemical determinations for the grade 0 group (6.5 ± 0 4) fell within the range of iron deficiency anemia. This group, however included 21 cases of iron deficiency anemia and 24 blood donors (together constituting 36% of the total) Since these subjects should by definition have empty iron stores, they could lower the mean substantially thus rendering it unrepresentative of a non-anemic population with no stainable reticular iron

The same analysis was therefore performed on 82 non anemic basal subjects. This showed that the means of the chemical determinations for the grades 0 to 2 + for the basal group did not

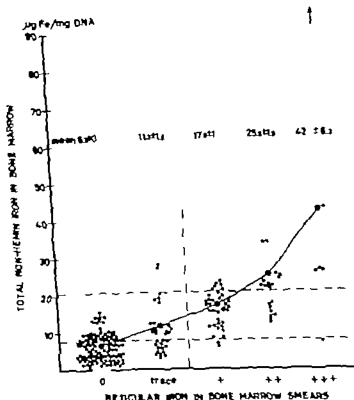


Fig. 28. Relation of non-hemin iron concentration in marrow to the grades of stainable reticulo-endothelial iron in smears.
The large dots denote the mean non-hemin iron concentration for the respective histochemic grade. The upper broken horizontal line is drawn through the mean non-hemin iron value for th control men, and the lower line through the upper limit for the group of iron deficiency anemia. Th figures are mean non-hemin iron concentrations with the standard errors for the respective histo chemical grade. See Table 29.

differ from the means for the respective histochemical grades for the whole material (Fig. 29).

In spite of the close correlation between the histochemical and chemical determinations there was a considerable overlapping (Fig. 28). For a fairly high percentage of subjects with no hemosiderin the chemically determined non hemin iron values were above the uppe limit for iron deficiency anemia. On th other hand, of the 100 subjects with a reticular hemosiderin grading of at leas 1 + *only 3* had non-hemin iron value within the range of iron deficiency anemia for only one of the 56 subject with chemically determined non hemi iron values above the mean for the mal

TABLE 29 *Analysis of the differences between the mean total non-hemin iron concentrations associated with 5 histochemical grades of stainable reticuloendothelial iron in smears. The analysis is performed on the subjects shown in Figure 28*

Stainable reticular iron Grade	n	Total non-hemin Iron in marrow (μg Fe/mg DNA) $\bar{X}$		Test of means* $\lvert t \rvert$	P
0	108	6.5 ± 4.6	$\bar{X}_1$	4.00 $(\bar{X}_2 - \bar{X}_1)$	< 0.001
trace	38	11.7 ± 7.5	$\bar{X}_2$	3.3 $(\bar{X}_3 - \bar{X}_2)$	< 0.001
1+	46	17.0 ± 7.0	$\bar{X}_3$	4.0 $(\bar{X}_3 - \bar{X}_4)$	< 0.001
2+	35	25.5 ± 11.0	$\bar{X}_4$	2.55 $(\bar{X}_4 - \bar{X}_5)$	< 0.05
3+	17	42.2 ± 25.9	$\bar{X}_5$		

* The means tested are in parentheses

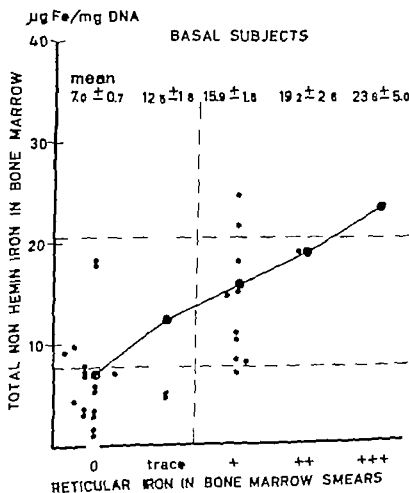


Fig 29 Relation of non-hemin iron concentration in marrow to the grades of stainable reticuloendothelial iron in smears from basal subjects. (See notes Fig 28)

control group did the smears contain no hemosiderin. It may be concluded that if reticular iron is present in the marrow smears, iron deficiency can be ruled out as the cause of anemia. Absence of reticular hemosiderin in the normal and mildly anemic subject indicates iron stores below the mean for control men but does not prove that the stores are exhausted.

The Prussian blue reaction in smear preparations and histological sections was evaluated by comparing the results of each method with the quantitative

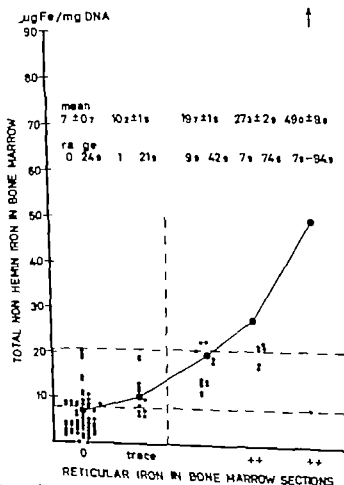


Fig. 30. Relation of non-hemin iron concentrations in marrow to the grades of stainable reticulo-endothelial iron in histological sections for 146 subjects in whom smears were examined simultaneously

TABLE 29 *Analysis of the differences between the mean t tal non-hemin iron concentrations associated with 5 histochemical grades of stainable reticuloendothelial iron in smears The analysis is performed on the subjects shown in Figure 28*

Stainable reticular iron Grade	n	Total non-hemin Iron in marrow (μg Fe/mg DNA) $\bar{x}$		Test of means[a] $\lvert t \rvert$	P
0	108	6.5 ± 4 6	$\bar{x}_1$	4 00 ($\bar{x}_2 - \bar{x}_1$)	< 0 001
trace	38	11 7 ± 7.5	$\bar{x}_2$	3.3 ($\bar{x}_3 - \bar{x}_2$)	< 0 001
1 +	46	17 0 ± 7 0	$\bar{x}_3$	4 0 ($\bar{x}_3 - \bar{x}_4$)	< 0.001
2 +	35	25.5 ± 11 0	$\bar{x}_4$	2.55 ($\bar{x}_4 - \bar{x}_5$)	< 0.05
3 +	17	42.2 ± 25.9	$\bar{x}_5$		

[a] The means tested are in parentheses

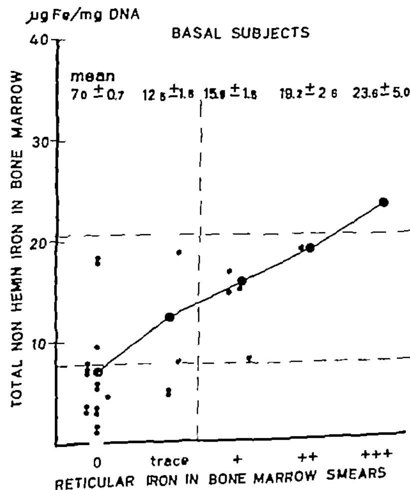


Fig 29 Relation of non-hemin iron concentration in marrow to the grades of stainable reticuloendothelial iron in smears from basal subjects. (See notes Fig. 28.)

rence between the chemical means for the groups with grade 0 and trace, but a highly significant difference between the groups with trace and grade 1+ whereas the reverse was the case for the smear technic.

Since for diagnostic purposes the presence of 1+ or more of reticular iron with either technic excludes iron deficiency as the actual cause of anemia, a finding of grade 0 or trace with one technic and 1+ or above with the other were deemed to be discrepant [191] Such discrepancies occurred in 26 of the 146 analysed cases, that is in 17%. When the histochemical estimations of these 26 cases were compared with the quantitative chemical ones the agreement was better for histological sections in 14 cases, and for smear preparations in 7 5 cases were inconclusive.

Sideroblasts

A coarse scatter diagram for the whole material showed that the sideroblast count was not correlated to stainable reticular hemosiderin or to the chemically determined non hemin iron. However most of the extremely low counts (≦12%) were associated either with absence of stainable hemosiderin or with non-hemin iron values within the range of iron deficiency anemia (0 to 7.7 µgFe/mg DNA) The exceptions were subjects with inflammatory disease or recent hemorrhage. From Fig 32, which shows the percentage of sideroblasts in different conditions, it is seen that only in iron deficiency anemia was the count consistently low with a mean of 2%/ and an upper limit of 12 / in the other groups there was a large scatter For the basal subjects the mean was 43% in 10 cases however the count was within the range of iron deficiency anemia (≦12%)

The blood donors who had recently recovered from iron deficiency anemia had a mean count of 27%. For most of those blood donors and those subjects with recent hemorrhage who were still slightly anemic the sideroblast count was within the range of iron deficiency anemia (Fig. 32)

The mean sideroblast count was lower in infection associated with hypoferremia (serum iron below 60 µg/100 ml) and higher in infection associated with normal serum iron than in the basal group In both groups sideroblast values within the critical range were encountered, although these subjects had a normal amount of stainable reticular iron, or a non-hemin iron value above the limit of iron deficiency anemia.

Since it was shown by the present and previous studies that the sideroblast count was influenced by (*i*) the presence or absence of excess iron in the body (*ii*) the availability of excess iron to the erythropoietic tissue at the time of assay and by (*iii*) inflammatory and toxic disease, the relation of the sideroblast count to other parameters of iron metabolism was further examined by regression analysis in 3 groups of subjects, in each of whom one of the above factors was relevant.

The relation of the sideroblast count to serum iron, transferrin saturation UIBC and non-hemin iron

BASAL SUBJECTS. — There was no correlation between the sideroblast count

chemical determinations. This was done on 146 subjects for whom the three assays were performed simultaneously. With both technics a highly significant correlation between the histochemical grading and the quantitative determination was obtained (Figs. 30 and 31). There was no difference between the section and smear technics as regards the *means* of chemically determined *non-hemin* iron for the respective histochemical grades.

The only difference between the two technics as regards the results of the chemical determinations was, that in the sections there was no significant diffe-

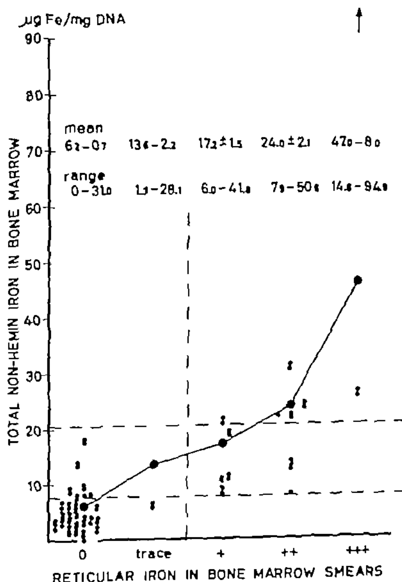


Fig. 31. Relation of non-hemin iron concentration in marrow to the grades of stainable reticulo-endothelial iron in smear preparations for the same subjects as in Fig. 30. (Notation as in Fig. 28)

rence between the chemical means for the groups with grade 0 and trace, but a highly significant difference between the groups with trace and grade 1+ whereas the reverse was the case for the smear technic.

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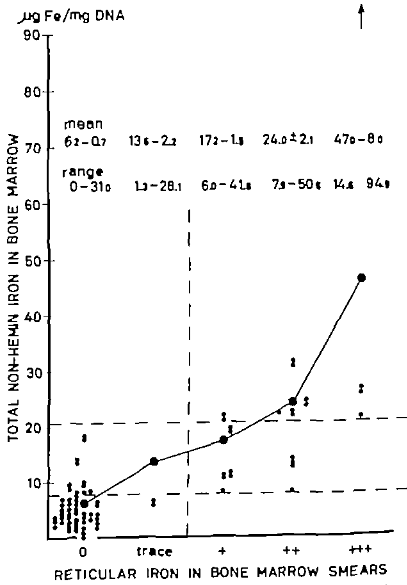


Fig. 31 Relation of non-hemin iron concentration in marrow to the grades of stainable reticulo-endothelial iron in smear preparations for the same subjects as in Fig 30. (Notation as in Fig. 28.)

rence between the chemical means for the groups with grade 0 and trace, but a highly significant difference between the groups with trace and grade 1+ whereas the reverse was the case for the smear technic.

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The relation of the sideroblast count to serum iron, transferrin saturation, UIBC and non-hemin iron

BASAL SUBJECTS. — There was no correlation between the sideroblast count

Table 30. *Characteristics of the groups shown in Figure 32*

	n	Hemoglobin (*g/100 ml*)	Serum Iron (*μg/100 ml*)	TIBC (*μg/100 ml*)	Non-hemin Iron (*μg F /mg DNA*)	Sideroblasts (%)
Basal subjects	84	14 1 *11.5–16.3*	142 *58–288*	330 *204–537*	12.3 *0–36 9*	43 *0–90*
Iron deficiency anemia	21	10.3 *5 9–12.6*	44 *14–121*	401 *336–625*	2.4 *0.0–7 7*	2 *0–12*
Blood donors non-anemic	20	14.3 *13 6–15.2*	115 *71–279*	390 *307–490*	5.2 *2.5–10 1*	34 *–64*
nemic	5	12.7 *11 7–13.2*	71 *44–106*	413 *348–454*	3.6 *1 7–6.1*	14 *0–50*
Recent hemorrhage	11	11 7 *8 7–16.2*	80 *44–160*	328 *241–397*	8.1 *1.9–14.3*	33 *0–94*
Infection serum iron						
< 60 μg/100 ml	13	10 9 *5 4–15 7*	45 *21–53*	252 *195–341*	23.6 *7.0–50.6*	8 *0–86*
> 60 μg/100 ml	25	11 6 *7.0–14 6*	111 *66–2?2*	279 *171–425*	27 8 *7.3–84.6*	54 *6–84*
Other anemias	11	7 4 *4.5–12.2*	160 *55–233*	244 *232 273*	40.8 *21.5–94.9*	76 *42–100*

Fig. 32. The sideroblast count in various groups. ● Non-anemic subjects Δ Anemic subjects. The horizontal lines denote the mean sideroblast percentage for th group

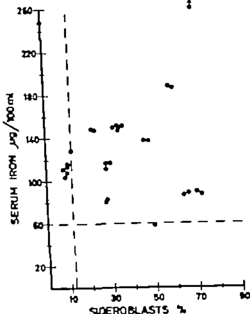
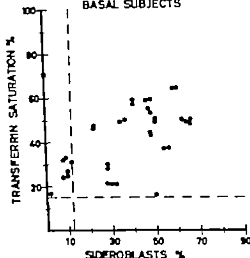
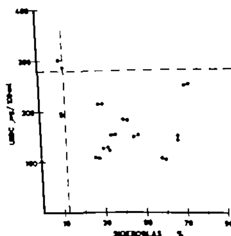


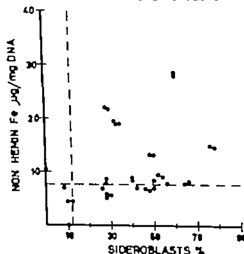
Fig. 33 A) Relation of the serum iron level to the sideroblast count in basal subjects.



B) Relation of the percentage transferrin saturation to the sideroblast count.



C) Relation of the unsaturated serum iron binding capacity to the sideroblast count



D) Relation of the total non-hemin iron concentration in marrow to the sideroblast count.

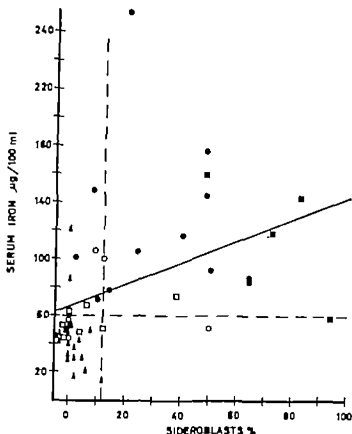
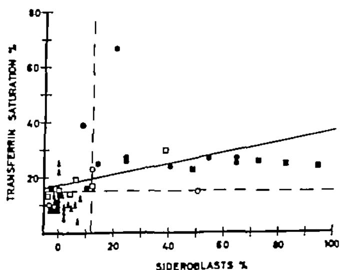
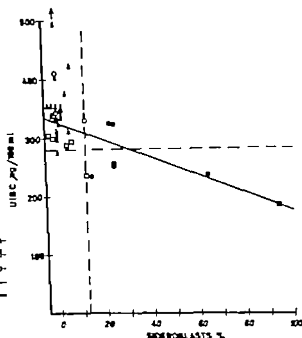


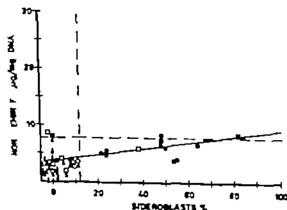
Fig. 34 A. Relation between the serum iron level and the sideroblast count in iron deficiency anemia (Δ), non anemic (●) and anemic (O) blood donors, and non anemic (■) and anemic (□) subjects after recent hemorrhage. $\bar{y} = 62.6 + 0.82\ x$ $n = 56$ $= 0.43$ $t = 3.47$ $P < 0.001$



B) Relation between the percentage transferrin saturation and the sideroblast count. $\bar{y} = 15.7 + 0.22\ x$ $n = 56$; $r = 0.45$ $t = 3.70$ $P < 0.001$



C) Relation between the unsaturated serum iron binding capacity and the sideroblast count. $\bar{y} = 335 - 1.62$; r = − 0.53 ; t = − 4.55 ; $P < 0.001$



D) Relation between the total non-hemin iron concentration in marrow and the sideroblast count. $\bar{y} = 3.6 + 0.054$; r = 0.43 ; t = 3.45 ; $P < 0.01$

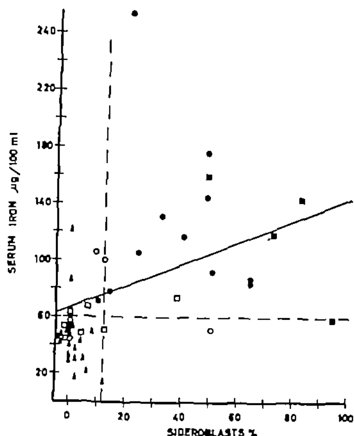
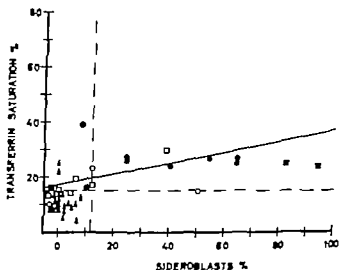


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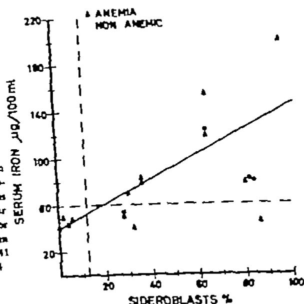
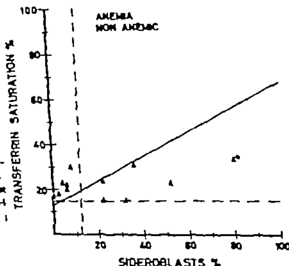


Fig 35 A) Relation between the serum iron level and the sideroblast count in infectious and toxic disease. The linear regression is calculated for only the anemic subjects in Figures 35A—35D $y = 41 + 1.03$; $= 24$ $= 0.64$ $t = 3.94$ $P < 0.01$



B) Relation between the percentage transferrin saturation and the sideroblast percentage $y = 13.2 + 0.54$ $= 0.65$; $= 4.02$; $P < 0.001$

and serum iron or the transferrin saturation (Figs. 33A—33B) The 10 subjects with counts of 0 to 12% had serum iron and transferrin saturation levels within the normal range. Nor was there any correlation between the sider oblasts and UIBC or non hemin iron *in marrow* (Figs 33C and 33D) Six of the 10 subjects with the critically low counts had a UIBC value above 280 μg/100 ml and 7 had a non hemin iron concentration within the range of iron deficiency anemia Two of the remaining 3 subjects were menstruating women and it is possible that the examination was performed just after their physiological blood loss.

Iron deficiency anemia blood donors and post hemorrhagic states.— The group comprised 56 subjects including cases of iron deficiency anemia blood donors most of whom had just recovered from iron deficiency anemia and had a small but statistically significant excess of non hemin iron in the marrow over that found in the subjects with iron deficiency anemia (see p 58) and cases of post hemorrhagic states with more or less depleted iron stores. The mean non hemin iron concentration for the whole group was 4.7 ± 0.4 μg Fe/mg DNA with a standard deviation of ± 3.16 Accordingly the group on the whole represented extremely low iron stores, with slight but distinct differences between the subgroups. Regression analysis showed that for this group, in contrast to the basal subjects, there was a significant correlation between the sideroblast count and serum iron, transferrin saturation, and non hemin iron and that there was a negative correlation between the sideroblasts and UIBC (Figs. 34A—34D)

All but 2 of the anemic subjects recorded sideroblast values within the critical range of 0—12% Only 4 non anemic subjects had such low counts. Of the 33 cases with counts within the critical range 9 had serum iron values above 60 μg/100 ml and 11 a transferrin saturation above 15% (Figs. 34A and 34B) On the other hand only 4 had an UIBC below 280 μg/100 ml and only 3 a non-hemin iron concentration above 7.7 μg/mg DNA (Figs. 34C and D) The last 3 subjects were cases of recent hemorrhage.

Infection and toxicity The subjects with hypoferremia and those with normal serum iron levels are treated together as a single group comprising 36 subjects, 24 of whom were anemic. A regression analysis performed for the whole group showed a significant correlation between the sideroblast count and each of the parameters studied Separate analysis of the anemic and non anemic subjects, however gave significant correlations only for the anemic group The regression lines in Figs. 35A to 35D were therefore calculated only for this group.

Critically low percentages of sideroblasts (0 to 12%) were encountered in subjects with normal as well as low serum iron and transferrin saturation values. On the other hand sideroblast counts above 20% were often associated with serum iron values below 60 μg/100 ml

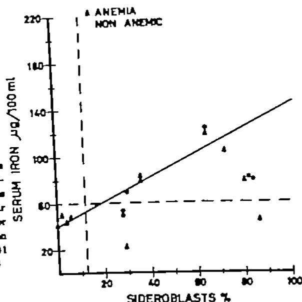
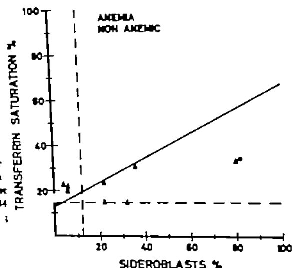


Fig 35 A) Relation between the serum iron level and the sideroblast count in infectious and toxic disease. The linear regression is calculated for only the anemic subjects in Figures 35A—35D $\bar{y} = 41 + 1.03 \bar{x}$; $= 24$; $= 0.64$; $t = 3.94$ $P < 0.01$



B) Relation between the percentage transferrin saturation and the sideroblast percentage. $\bar{y} = 13.2 + 0.54 \bar{x}$; $= 0.65$; $= 4.02$; $P < 0.001$

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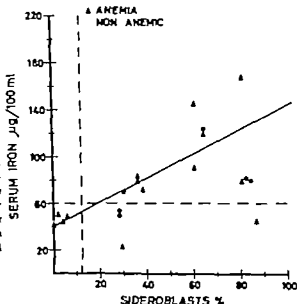
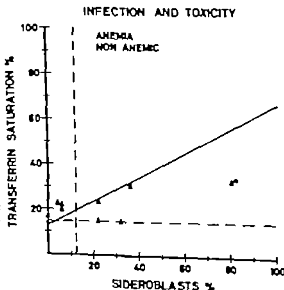
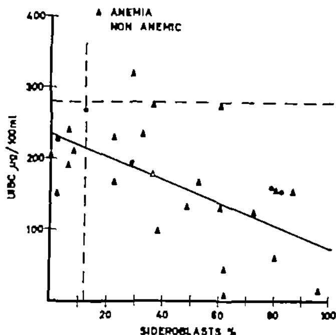


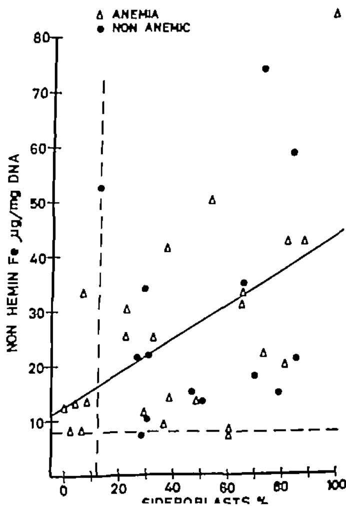
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B) Relation between the percentage transferrin saturation and the sideroblast percentage. $\bar{y}$ = 13.2 + 0.54 = 0.65 ; = 4.02 $P<0.001$



C) Relation between the unsaturated serum iron binding capacity and the sideroblasts count. $\hat{y} = 234 - 1.6x$ $r = -0.58$; $t = -3.34$; $P < 0.01$



D) Relation between the concentration of total non hemin iron in marrow and the sideroblast count $\hat{y} = 11.1 + 0.34x$; $= 0.55$ $t = 3.06$; $P < 0.01$

Comments

In the normal subject the sideroblast count showed a wide scatter and varied independently of the other parameters of iron metabolism examined. The count assumes significance, however when it has fallen to critically low values — from 0 to 12 per cent. This range was encountered in all untreated cases of chronic iron deficiency anemia. Most of the basal subjects with a count within this low range also had non-hemin iron concentrations within the range of iron deficiency anemia, with a fairly high UIBC, but normal serum iron and transferrin saturation. These results, together with the fact that for the basal group no correlation was found between the sideroblast count and the non-hemin iron concentration, are consistent with the assumption that only a small excess of iron is necessary to provide a normal sideroblast count. The results of the correlation analysis performed on the basal subjects also indicate that in the course of iron depletion the sideroblasts disappear before the serum iron and the transferrin saturation have fallen below the normal range. On the other hand, the significant negative correlation between TIBC and non-hemin iron concentration for the basal group indicates that the TIBC rises at an earlier stage of iron depletion than the sideroblast count falls. Thus, although there was no correlation between the sideroblasts and the TIBC or UIBC in the basal group, most of these subjects having a critical sideroblast count had a fairly high TIBC.

The analysis of the group comprising blood donors and cases of iron deficiency anemia and recent hemorrhage further confirms the assumption that the iron pool responsible for the normal sideroblast count is small and labile. In this group there was a significant correlation between the count and each of the parameters examined. This is to be expected in view of the fact that the group consisted of subjects in whom there was either no excess of iron, or the excess was on the borderline of what is required to maintain a normal count. With the exception of 3 subjects, all in this group who had critically low sideroblast values had *non-hemin iron concentrations* within the range of iron deficiency anemia (Fig. 34 D). The 3 exceptions were cases of recent hemorrhage, 2 of them still anemic. This is consistent with the conclusion reached in previous studies that when there is an increase in the demand for iron, as after hemorrhage or B_{12} therapy in pernicious anemia, the readily available sideroblast iron pool is consumed, and the rate of mobilization of excess iron may not suffice to give a normal sideroblast count. This is not always the case and probably depends on the availability of iron in the stores and the gut, on the one hand, and the rate of hemoglobin formation on the other.

From a comparison between the iron deficiency anemia group and the non-anemic blood donors in respect of the mean non-hemin iron concentrations in marrow and sideroblast counts, the *normal sideroblast* iron pool has been coarsely estimated at about 45—90 mg of non-hemin iron (see p. 62). The extensive ferrokinetic studies by Polly

cove and Mortimer [193 194] disclosed the existence of two distinct labile iron pools, the larger one associated with erythropoiesis and the smaller with storage. These authors found the mean erythropoietic labile iron pool to be 85 mg of non-hemin iron in the normal and 10 mg in iron deficiency anemia The present estimates of the sideroblast iron pool in these conditions are in agreement with the above figures. Hence, a hypothesis is advanced that the sideroblast iron pool constitutes a large fraction of the labile erythropoietic iron pool. It is notable that the labile erythropoietic iron pool determined by Pollycove in subjects after hemorrhage and in pernicious anemia following 4—7 days treatment with vitamin B_{12} was usually greatly depressed, whether or not storage iron was present and was correlated to the serum iron level [195] Previous findings [124] and the present results as regards the sideroblast count in these conditions were similar The labile erythropoietic iron pool and the sideroblast count are dependent not only on the presence of excess iron but also on its availability and they will therefore tend to decrease in conditions involving *accelerated production of hemoglobin* On the other hand, in pernicious anemia in relapse and sideroachrestic and refractory anemias the labile erythropoietic pool is increased on the histochemical evidence of a high percentage of iron laden sideroblasts this would indicate an increased sideroblast iron pool. In hypoplastic anemia despite the larger number of granules in the sideroblasts, the cell population itself is small, and actually *corresponds to a low* labile erythropoietic iron pool [193]

In the conditions discussed above the fall in the sideroblast count was due either to an absence of excess iron in the body or to the inability to mobilize iron at a rate fast enough as to provide an excess for the formation of sideroblasts. The cause of low sideroblast counts in some cases of infection and inflammatory disease is more complex.

In anemia of infection significant correlations were found between the sideroblast count and the serum iron, the *transferrin saturation, the UIBC and* even between the concentration of non-hemin iron in the marrow although the storage values were usually above the upper limit of iron deficiency anemia (Figs. 35 A—D) However on comparing the curves for the infection group (Figs 35 A and 35 B) with those for the *iron depletion group* (Figs. 34 A and 34 B) a difference is apparent. In the latter all but 2 subjects with a serum iron level below 60 μg/100 ml had a critical sideroblast count of ≦ 12 per cent moreover when the subjects with serum iron levels below 60 μg/100 ml were excluded there was no correlation between serum iron and sideroblasts for the rest of the subjects (Fig 34 A) The same was the case for the transferrin saturation and sideroblasts, there being no correlation if subjects with a saturation below 16 per cent were excluded. (Fig 34 B) On the other hand in the group of *anemia* of infection many subjects with extremely low serum iron had a sideroblast count within the normal range. *Moreover* the figures show that

in contrast to the former group the significance of the correlations between the sideroblasts and the parameters examined is not dependent on the presence of subjects with low serum iron and low transferrin saturation values (Figs. 35 A—D). In the last group a correlation was found between the sideroblasts and the other parameters over the whole range of values and even if the subjects with serum iron below 60 μg/100 ml were excluded.

It may be inferred, then, that in the first group with depleted iron stores an abnormally low serum iron level and transferrin saturation indicate exhaustion of iron and is therefore consistently accompanied by a critical sideroblast count. The findings for the second group could be explained by assuming the presence of a factor in anemia of infection that depresses the serum iron level and the sideroblast count simultaneously. There need then be no direct relation between the serum iron and the sideroblast count, both of them being dependent on this factor. Its tendency to lower both the serum iron and the count might on some occasions be offset by other factors, such as enhancement of hemolysis, that would tend to increase both parameters.

The presence in anemia of infection but not in the basal group of a significant correlation between the sideroblast count and the non-hemin iron in marrow [Fig. 35 D] may have the following explanation. In the basal group the sideroblast count displayed random variations, unrelated to the stores, because most subjects had enough excess iron to provide a normal count. In anemia of infection, however, the activity of the disease, which has a tendency to lower the sideroblast count, might be moderated by the size of the iron stores, the fall in the count being smaller in subjects with well filled than depleted stores. The correlation between stores and sideroblast count in anemia of infection could, however be due to another factor — in addition to the one tending to lower the count — that has the opposite effect on the count and that at the same time tends to augment the iron stores. Such a factor might be enhanced hemolysis. According to this interpretation the stores *per se* would not influence the sideroblast count.

Another explanation of the low sideroblast count in anemia of infection has recently been advanced by Bainton and Finch [196]. They found that in a combined group of iron deficiency anemia and anemia of infection the sideroblast count was more closely correlated to the percentage saturation of transferrin than to the serum iron level. From the early findings of Jandl *et al* [181] that the uptake of iron by reticulocytes *in vitro* was a function not only of the iron concentration but also of the transferrin saturation Bainton & Finch conclude that the percentage saturation of transferrin may be considered as an index of iron supply to the marrow. When the saturation of transferrin falls below 16 per cent the supply of iron to the erythroid marrow appears to be inadequate, and when this condition persists for long enough "iron deficient erythropoiesis" with microcy

cove and Mortimer [193 194] disclosed the existence of two distinct labile iron pools, the larger one associated with erythropoiesis and the smaller with storage These authors found the mean erythropoietic labile iron pool to be 85 mg of non hemin iron in the normal and 10 mg in iron deficiency anemia. The present estimates of the sideroblast iron pool in these conditions are in agreement with the above figures. Hence, a hypothesis is advanced that the sideroblast iron pool constitutes a large fraction of the labile erythropoietic iron pool. It is notable that the labile erythropoietic iron pool determined by Pollycove in subjects after hemorrhage and in pernicious anemia following 4—7 days treatment with vitamin B_{12} was usually greatly depressed, whether or not storage iron was present and was correlated to the serum iron level [195] Previous findings [124] and the present results as regards the sideroblast count in these conditions were similar The labile erythropoietic iron pool and the sideroblast count are dependent not only on the presence of excess iron but also on its availability and they will therefore tend to decrease in conditions involving accelerated production of hemoglobin On the other hand, in pernicious anemia in relapse and sideroachrestic and refractory anemias the labile erythropoietic pool is increased on the histochemical evidence of a high percentage of iron laden sideroblasts this would indicate an increased sideroblast iron pool.. In hypoplastic anemia, despite the larger number of granules in the sideroblasts, the cell population itself is small and actually corresponds to a low labile erythropoietic iron pool [193]

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ciency as the actual cause of anemia. Absence of stainable hemosiderin in the individual subject does not prove that the iron stores are exhausted. Absence of sideroblasts in a subject in steady state indicates that there is no excess iron. If hemosiderin is present and sideroblasts are absent either inflammatory disease or recent hemorrhage should be suspected.

tosis and hypochromia would develop whether or not there is storage iron They consider that the *same applies* to the sideroblast count According to this theory the ultimate cause of the low sideroblast *count in infection would be* the same as in iron deficiency i e inadequate iron supply to the marrow because of a decrease in the transferrin saturation the authors point out however *that disturbance of hemoglobin* formation for reasons other than deficient iron supply may alter the sideroblast pattern and then the sideroblast count would not be related to the *percentage saturation of transferrin* This interpretation is essentially different from that suggested above namely that in infection the serum iron and transferrin saturation on the one hand, *and the sideroblast count on the other* are dependent on a third factor and not necessarily interdependent.

The results of the present study however are not consistent with the hypothesis of Bainton and Finch for the following reasons (*i*) The presence of a significant correlation between the *per cent saturation of transferrin and* sideroblast count in cases of infection together with the fact that there was no such correlation for the basal group with transferrin saturations of a similar range (Fig 33 B and 35 B) indicate that the low sideroblast count in infection was not due to an inadequate supply of iron *via* the plasma. (*ii*) There was no difference between the correlation coefficients for the regressions of serum iron and sideroblasts on the one hand *and per cent* saturation of thansferrin and sideroblasts on the other when the iron deficiency and the infection groups were analysed separately (Figs 34 A—B 35 A—B) *The view* that the supply of iron to the erythron *is dependent* on the per cent saturation of thansferrin has recently been refuted [197 198] (*iii*) The positive correlation between the non hemin iron concentration in marrow and the sideroblast count found in anemia of infection but not in the basal group suggest that the sideroblasts derive their iron not only from the plasma but also from other sources — perhaps directly from the reticulum cell by "rhopheocytosis" as suggested by Bessis [41] Our results indicate that the cause of a low sideroblast count in some types of infection is more complex than in the case of iron *deficiency where it is due solely to the* absence of excess iron in the body

The frequency with which "critically" low sideroblast counts are encountered in anemia of infection differs according to the material In one study about 10 per cent of the cases of anemia of infection had such low values [124] and in the present material the figure was about 25 per cent. The difference *is* probably due to the composition of the material Extremely low counts appear to be associated with active *rheumatoid* arthritis and Hodgkin s disease more often than with other infectious conditions [199]

For diagnostic purposes the results can be summarized as *follows Stainable* reticular iron in smear preparations and in histological *sections of* grade 1 + or above rules out iron defi

Summary

The prime object of this investigation was to determine the non-hemin iron and ferritin iron in liver and bone marrow of healthy adults and in states of iron depletion and other pathologic conditions, and to examine the possibility of a relationship between, on the one hand, the non-hemin iron concentration and, on the other the total iron-binding capacity (TIBC) and serum iron.

Total non-hemin and ferritin iron (water-soluble iron) were determined chemically in surgical liver biopsy specimens and in bone marrow aspirates. Parallel with this determinations were made of the serum iron and total iron binding capacity and reticuloendothelial iron and sideroblasts were assayed by histochemical techniques. The non-hemin iron in marrow was determined on the basis of DNA, which was estimated microbiologically.

The material of 302 subjects included in this investigation consisted of control, basal and pathological groups. The controls are defined as hematologically normal with no history of non-physiological blood loss in the past. The "basals" were hematologically normal subjects with no signs of infection and no history of hemorrhage, at least during the year prior to the study. The pathological groups were divided into two major groups: one consisted of cases of disturbed iron metabolism, with the sub-groups iron deficiency anemia, hemorrhage, blood donors and subjects that had undergone partial gastrectomy. The second major group comprised heterogenous diagnoses, classed as "states of infection and toxicity". This group was subdivided with respect to factors affecting the iron stores and iron metabolism.

Control groups. — The concentration of *total non-hemin iron* in both liver and bone marrow was significantly higher for the male group than for the non-menstruating women. As regards the liver there was also a statistical difference between the non-menstruating and menstruating women; in the marrow the difference between non-menstruating and menstruating women was not significant.

The concentration of *ferritin iron* (water soluble iron) in liver was significantly higher for the control men than for the combined group of women and almost significantly higher than for non-menstruating women. The mean for the latter was higher than for menstruating women (almost significant).

As regards the *proportion of ferritin iron* in total non-hemin iron there was no significant difference between the three control groups.

Sequence of Events in Iron Depletion

On the basis of the results of this investigation it would appear that iron depletion in the non anemic, non hemorrhagic subject involves the following sequence of events.

The normal subject with a non hemin iron concentration of 80 mg/100 g dry weight, in the liver and 20 6 μg Fe/mg DNA in the marrow has, on an average a TIBC of 280—290 μg/100 ml, a serum iron level of 140 μg/100 ml, and a transferrin saturation of 47—50 per cent. He will usually have stainable reticuloendothelial iron in the marrow with a histochemical grade of 1+ or more, and a sideroblast count of about 50 per cent When the storage iron falls to 29 mg/100 g, dry weight of liver tissue, or to 7.5 μg Fe/mg DNA in the marrow the TIBC rises on the average to 335—345 μg/100 ml. There will be no significant change in the serum iron level or the saturation of transferrin. The marrow will usually not contain stainable reticuloendothelial iron The sideroblast count remains unchanged

With further depletion of the iron stores the TIBC continues to rise while the serum iron and saturation of transferrin remain on the average constant.

There is a tendency however towards a greater dispersion of the values for serum iron and transferrin saturation.

The sideroblast count is still unchanged

When the storage iron has been practically all consumed and the non-hemin iron of the total red marrow has fallen approximately below 40 mg the sideroblast count will steadily decrease and with further diminution of the excess iron, it will fall to the critical values of 0—12 per cent encountered in iron deficiency anemia. This will occur before the serum iron has reached subnormal levels. The TIBC will then be on an average, 380 μg/100 ml With further restriction of the iron, the serum iron level and the saturation of transferrin will decrease, the iron supply to the erythroid marrow will become insufficient, the formation of hemoglobin will be retarded and iron deficiency anemia will ensue.

four cadavers varied appreciably but there was a relationship between the values for different sites in the same subject. In all subjects the sternal marrow had the lowest concentration.

For the basal subjects there was a highly significant correlation between the non-hemin iron concentrations in liver and bone marrow. The scatter of the individual values was appreciable, however, and the correlation coefficient accounted for only 52 per cent of the variation. For a group with liver damage and jaundice, comparison with the basals disclosed a significantly higher concentration of non-hemin iron in the marrow than expected from the liver values. In recent hemorrhage there was a lower concentration in the marrow. The above groups were however small. In the other groups with infectious or toxic disease the mean concentrations were slightly higher in the marrow than expected from the liver values, but not significantly different from those for the basal group.

In both liver and marrow of non-anemic hematologically normal subjects there was a negative highly significant correlation between the *TIBC* and *iron stores*. This relation was curvilinear and the slope of the regression line was greater for subjects with low storage iron values. From comparison of the pathological groups with the basal subject it was evident that the TIBC values were higher for the group of recent hemorrhage and blood donors and significantly lower for the groups of anemia of infection and megaloblastic anemia than was expected from the equation for the basal subjects. The results indicate that the iron stores are the basic regulator of the transferrin level in serum, although this may be affected independently by other factors such as for instance disturbance of the protein metabolism.

In the non-anemic basal subjects no correlation was found between the *iron stores* and the *serum iron* level or the *saturation of transferrin*. The results suggest that when the iron stores are low there is a rise in the serum iron in some of the subjects so that in spite of a high transferrin level the mean saturation of transferrin remains unchanged.

The presence of at least grade 1+ of reticuloendothelial iron, estimated by either the *smear* or histological *section* technic, rules out iron deficiency as the actual cause of the anemia. Herein lies the main diagnostic value of the method. Although suggestive of subnormal iron stores, the absence of hemosiderin does not provide a measure of the severity of the deficiency and is therefore not a reliable diagnostic sign of iron deficiency anemia, especially if the anemia is mild.

There was a highly significant correlation between the histochemical grades of reticuloendothelial iron and chemically determined non-hemin iron, but with a considerable degree of overlapping. Thus, if the method is applied fo examination of a group of subjects the histochemical grade of stainable reticuloendothelial iron will provide a reliable estimate of the state of their iron stores.

The amount of excess iron needed to give a normal sideroblast count is small. The sideroblast iron pool for the normal

In none of the adult groups of either sex was total non hemin or ferritin iron in the liver correlated to age As regards the bone marrow there was a significant correlation between total non hemin iron and age in the male group this was due mainly to a rise in the ages up to 55 years

The proportion of ferritin iron was not correlated to age.

The storage iron values for apparently normal adult men without hemorrhage showed a fairly large scatter

States of iron depletion — For the group of *iron deficiency anemia* the concentrations of total non hemin and ferritin iron in the liver and bone marrow were significantly lower than for all 3 control groups. There was no overlapping between the values encountered *in control males and those of iron deficiency anemia* There was however a considerable degree of overlapping between the values of control women and non anemic subjects with depleted iron stores on the one hand and iron deficiency anemia on the other

In cases of iron deficiency anemia and in non anemic subjects *with well defined* iron depletion, such as blood donors, there was a low scatter of the storage iron values The absolute difference between the mean non hemin iron concentration in iron deficiency anemia and that of *the blood donors* who had recently recovered from iron deficiency anemia was small but highly significant. The fact that the blood donors had at the same time a higher serum iron level and sideroblast count than the anemic subjects, would indicate that only a small excess of iron is necessary to raise the serum iron and the sideroblast count, and that after recovery from anemia this amount is soon replaced by enhanced uptake from food. On the other hand that the iron stores were not significantly different in subjects with old hemorrhage from those with recent hemorrhage suggests a slow replenishment of the stores.

Infection and toxicity — In *infectious* and *toxic* non-hemorrhagic disease, whether in the absence of anemia or with mild or moderate anemia, the concentrations of total non-hemin and *ferritin iron in the liver and bone marrow* did not differ significantly from the means for the respective control groups. The only groups with significantly higher storage iron values than in controls were that of severe megaloblastic anemia and a miscellaneous group of *infectious and toxic diseases* who had received an exogenous load of iron.

The mean *proportion of ferritin iron* in total non hemin iron in the liver of the basal subjects was 60 per cent, over a *range of* total non hemin iron con*centration from 4 to 227 mg per 100 g* dry weight. There was a tendency towards a greater dispersion of the values for high total non hemin iron concentration Of the pathological groups only those of recent hemorrhage had considerably lower values than the basal group The groups of anemia of infection — *even* that with fairly high iron stores — did not differ from the basal subjects.

The concentration of non hemin iron in marrow from different *flat bones of*

Appendix

TABLES

individual has been coarsly estimated at about 45—90 mg of non-hemin iron which is about 10—25 times the plasma iron It is suggested that the sideroblast iron pool constitutes a large part of the labile erythropoietic iron pool

In the basal subject the sideroblast count shows a large scatter There is no correlation between the sideroblast count and the non-hemin iron concentrations in the marrow for the basal subjects. This is ascribable to the fact that little iron in excess is needed to give an optimal count for the individual The *sideroblast count assumes significance* when it drops to critical values of 0—12 per cent as is invariably the case in chronic iron deficiency anemia. Such a low count indicates that there is practically no excess iron in the body Exceptions from this rule are conditions in which there is enhanced formation of *hemoglobin* as *after hemorrhage or* during administration of vitamin B_{12} for pernicious anemia. In such situations the sideroblasts may temporarily vanish because of an inability to mobilize iron at a rate high enough to provide an excess of iron for the formation of sideroblasts. Another exception is infectious and *toxic disease, especially those cases* with fever and active inflammation, in whom the sideroblast count may drop to critically low values in spite of the presence of storage iron. In infection a significant correlation was found between serum iron, transferrin saturation *and non hemin iron, on the one hand,* and sideroblasts on the other It is possible that in inflammatory disease there is a factor that lowers both the serum iron level and the sideroblast count. The fall in the count does not seem to be directly caused by the lowered serum iron or percentage saturation of trans*ferrin. It is rather more probable that* all these parameters are dependent from another factor active in anemia of infection and toxicity

A determination of reticuloendothelial iron combined with a sideroblast count is recommended as a diagnostic method. Absence of stainable reticulo*endothelial iron together* with a critical sideroblast count indicates complete exhaustion of excess iron The presence of stainable iron rules out iron *deficiency* as the actual cause of anemia. The presence of reticuloendothelial iron and the absence of sideroblasts suggests either recent hemorrhage or inflammatory disease.

Non-hemin iron in surgical liver biopsy specimens			N h mlo l l bo m ow plrates						
Total non-hemin iron (mg Fe/100 g liver)		Ferritin iron (mg/100 g)	Chemical determination (μg Fe/mg DNA)			Stainable iron			
			Total non-hem iron		Ferritin			Sideroblasts	ESR
wet wt	dry wt	dry wt	uncorr	corr.	corr	Smears	Sections	(%)	mm/h
18.5	61.9	35.7	21.6	21.2			1+		6
16.6	55.4	37.4	25.2	24.9		1+	0	36	9
36.2	120.7	66.7	23.9	21.9	22.6	2+	1+	34	13
15.1	41.6	16.1				trace		42	4
26.1	88.7	53.9							6
18.1	61.4		11.0	10.1			1+		
30.8	102.9	43.7				2+	2+	84	5
33.2	111.1	58.9							7
34.0	113.6	62.9				3+	3+	85	2
18.1	60.3	25.2				trace	3+	54	
9.1	30.4	13.0				trace	1+	86	15
20.9	69.7	31.6				trace	0	84	
42.3	141.2	67.8					1+		
14.1	47.0	14.9				trace		44	11
21.9	73.2	63.7	18.7	18.4		0	trace	16	4
68.0	227.0	174.4	36.7	36.9		3+		45	4
15.1	50.4	31.6	22.0	20.3		trace	1+	58	3
14.6	48.8	34.4	13.6	13.2	11.4	1+	1+	48	11
36.6	124.4	83.8				2+		84	6
21.8	74.0	48.9	21.9	21.9	20.6	1+	trace	28	8
36.5	107.1	72.2							10
5.7	19.4	10.8	18.9	18.9		trace	trace	33	6
	54.8								
22.5	75.0					2+	2+	60	16
18.8	62.8	55.4	13.5	13.5		0		50	58-38
25.4	84.8	42.9	7.6	7.0		1+		23	20
18.4	61.1	40.2	33.3	33.7	30.5	2		28	80-15
28.8	104.7	71.9							14
20.4	61.5	36.4	23.4	21.9		1+	0	30	17
3.6	11.9	5.5	4.9	4.4		0	0	48	19
14.0	46.6	33.3	6.8	6.8		0	0	48	
15.2	50.7	39.8	13.2	13.1		2+	trace	50	9
30.1	106.6	64.3	22.9	22.5	19.0	1+	1+	80	5
22.4	73.3	40.2	15.4	14.8		trace		50	
	44.4								21
5.7	19.7	11.1	7.1	6.3		0		10	18
27.0	90.1	54.3				2+	2+	55	22
21.0	70.1	48.4	15.0	15.0		2+		78	19
9.2	31.2	20.6							
11.1	34.1	24.5	12.0	8.7					14
1.7	5.5	4.6	0.0	0.0	0.0	0	0		
4.7	14.0	14.9	10.8	10.5		trace		10	
7.0	26.9	20.9	11.6	10.5	10.4	0		82	11
10.2	31.1	23.7	8.2	7.9		trace	0	0	12
7.3	24.7	13.3						28	5
									12

Subject		Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum Iron (μg %)	TIBC (μg %)
1	F N	M 41	B[1] + C[2]	Uncompl. gastric ulcer No hemorrhage	15.5	128	265
2	J P	62	"		14 0	151	305
3	F G	62	"	"	13.5	91	299
4	B S	24	"	"	14.3	112	287
5	J S	44	"	"	14 0	183	336
6	I B	31	"	"	13.8	168	2 6
7	F F	50	"	"	16.6		
8	T A	38	"	"	15.3		
9	E E	32		"	13.7		
10	A H	39	"	"	15.2		
11	H N	39	"	"	16.8		
12	A H	42	"	"	14.2		
13	O K	61	"		14 1		
14	E H	61	"	"	13 9		
15	T L	34	"	Uncomplicated cholelithiasis	15.2	130	305
16	G G	57	"	"	15 1	120	269
17	A R	37	"		16.1	187	293
18	R N	45	"		13.6	123	287
19	A L	50	"	"	13.6	126	38
20	P G	59			13.5	82	296
21	A G	66	"	"	14.2	198	253
22	G H	59	"	"	14.5	173	296
22 b	P A	53			14 1		
23	B A	41	Inf[3] + C	Chronic cholecystitis	16.1		
24	P O	38	"	"	15.7	52	265
25	H H	27	"	Acute cholecystitis	15 1	49	281
26	C R	77	"	Chronic cholecystitis	13.7	53	24
27	L R	32	"	Chronic and acute cholecystitis	15 4		
28	H L	27		Chronic cholecystitis	13.5	119	340
29	N S	F 72	B + C	Uncomplicated cholelithiasis	14 9	137	317
30	O M	77	"	"	16.0	137	253
31	B V	51			11 9	113	29
32	S C	58			13.0		
33	N S	46	"	"	12.6	151	305
34	L S	48	"	"	14.5		
35	G G	56		Uncomplicated gastric ulcer	11 7		
36	S J	62	Inf + C	Chronic cholecystitis	13.8		
37	H H	57	"		13.2	148	308
38	K H	66	"	"	13.5	214	284
39	B H	63		"	14 5	115	353
40	M M	Fm 46	B + C	Uncomplicated cholelithiasis	13.7	108	433
41	S K	35		Biliary dyskinesia	12.4	123	311
42	W E	23	"	Uncomplicated cholelithiasis	11 8	249	331
43	F S	35			13.0	198	305
44	K B	37	"	"	14.6		

[1] Basal subject [2] Control subject [3] Infectious or toxic state without anemia

Fm = Menstruating women

Non-hemin iron in surgical liver biopsy specimens			Non-hemin iron in bone marrow aspirates: Chemical determination (µg Fe/mg DNA)			Stainable iron			
Total non-hemin iron (mg Fe/100 g liver)		Ferritin iron (mg 100/g)	Total non-hem iron		Ferritin			Sidero-blasts	ESR
wet wt	dry wt	dry wt	uncorr.	corr	corr.	Smears	Sections	(%)	mm/h
1 2	4.0	0 0							20
	21.5								13
	37.7								8
19 4	65.9	21.6	18 4	17 1		1+		48	17
24 9	83.2	58.4	10 1	10 1		trace		30	35
8 8	29.2	21.3							23
8.7	29 4	19 7							82
2.2	7.3	4 5	6 2	5.0					
2.8	10 7	6.7				trace		15	39
20.4	68 1	48.0				2+	1+	21	
45 8	206.5	84 0	47.0	46 7					10
15 2	51 5	31.7				0	0	6	39
17 1	58.0	32.9							3
23 0	64	40 8	21.3	21.3	16.7	3+	3+	64	17
30 5	105 2	40.2	22.5	22 4		2+	1+	48	20
24 4	81.5	46 3	43 0	43 0	23 0	2+	2+	80	7
83.5	279.0	130 8	34 2	33 8	22.4	2+	2+	64	29
14 6	48 8	32 1	17 9	18 1	12.4	1+	1+	0	27
41 0	136 8	92 2	27.5	26 7					52-22
36 7	176.6	79 1							94-28
10 8	36 1	23 8	14 1	13.8		0		8	14
11.8	33 3	27 6	31 4	31.3	31.3	0	2+	64	111
35 2	148.6	100 5	50.0	50 6	41.9	+	2+	32	92
14 9	50 6	24 6					1+		15
5 6	18.6	11 7				trace		40	60
10 6	32.2	16 5							44
9 1	30 4	20 2	7 8		6 1	trace	0	6	58
4 9	16 4	11 3	14 0	13.6		1+	1+	48	26
20 6	61 8	54 9	29 0	30 5		2+	1+	22	58
7 1	21 9	15.4				1+	1+	5.	59

Subject		Sex and age	Group	Clinical diagnosis	*Hb* (g %)	Serum iron (μg %)	TIBC (μg %)
45	S B	Fm 19	B[1] + C[2]	Uncomplicated cholelithiasis	12.1		
46	A J	31	"	"	13.6		
47	B G	17	"	"	14 0		
48	J B	43	"	Uncomplicated gastric ulcer	15.3	177	299
49	J I	34	Inf.[3] + C	Chronic cholecystitis	14 4	69	273
50	K A	33	"	"	12.7	153	394
51	U I	34	"	Chronic and acute cholecystitis	11 6	117	314
52	Å E	42	"	"	12.8	160	414
53	S G	M 47	Inf + *Cirrh.*[4]	Uncomplicated gastric ulcer and cirrhosis hepatis alcoholica	14.2		
54	J R	54	"	Uncomplicated gastric ulcer and cirrhosis hepatis alcoholica	13.5	69	284
55	H P	49	Inf	Uncomplicated gastric ulcer Chronic hepatitis with moderate portal fibrosis. No change in structure	17 7	227	361
56	R A	52	"	Chronic cholecystitis and colitis UNS	14 2	100	333
57	I B	37	Inf + Jaun.[5]	Chronic cholecystitis. Steatosis hepatis alcoholica with jaundice	14 8	217	347
58	J G	63	"	Chronic cholecystitis with obstructive jaundice	13.8	79	28
59	S J	66	"	Chronic ch lecystitis with obstr. jaun.	14 7		
60	K V	46	Inf + Meg a.[6]	Chronic cholecystitis and pernicious anemia in relapse	11 9	78	235
61	O W	63	A. of inf[7]	Cancer of the pancreas with metastases	10.8	154	193
62	S S	63	"	Pyloric stenosis. Steatosis hepatis	11 4	41	244
63	S I	36	A of inf + Jaun.	Chronic cholecystitis and acute pancreatitis with obstructive jaundice	13.3	136	421
64	V M	67	A of inf	Chronic and acute cholecystitis	12.7	107	295
65	A O	6	"	Chronic cholecystitis and mediastinal lymphadenosis. Chronic subfebrility	13 1	91	302
66	A S	73	A of inf + Jaun.	Severe biliary cirrhosis with jaundice	10.5	118	125
67	O E	70	"	Cholangiogenic hepatitis with severe jaundice	12.7	50	217
68	G S	F 61	A of inf.	Chronic cholecystitis	10 9	146	280
69	H M	57		Chronic cholecystopathy and biliary fistula	10 7	202	296
70	P A	56		Hepatic cyst with a sterile abscess. Cholecystopathy	10 0	46	268
71	S E	79		Rheumatoid arthritis and chronic cholecystopathy	9 6	66	306
72	B L	50		Uncomplicated gastric ulcer and anemia of infection	10 1	143	75
73	A A	80	A. of Inf + transfusions	Chronic cholecystitis and subacut pyelonephritis	9 4	42	27
74	N E	63	Inf. + Jaun	Biliary cirrhosis of the li er and choledocholithiasis with jaundice	11 4	107	339

[1] Basal subject [2] Control subject [3] Infectious or toxic state without anemia [4] Cirrhosis of the liver [5] Jaundice [6] Megaloblastic anemia [7] Anemia of infection or toxicity
Fm = Menstruating women

Non-hemin iron in surgical liver biopsy specimens			N h ml l o l bon m w pl tes						
Total non-hemin iron (mg Fe/100 g liver)		Ferritin iron (mg/100 g)	Chemical determination (μg Fe/mg DNA)			Stainable iron		Sidero-blasts	ESR
			Total non-hem iron		Ferritin				
wet wt	dry wt	dry wt	uncorr.	corr.	corr.	Smears	Sections	(%)	mm/h
13.8	*46.8*	33.3				1+	2+	50	58
3.7	*12.4*						0	32	2
23.0	*76.7*	26.3				1+	1+	60	3
5.1	*17.0*	0.6				1+	0	74	2
8.2	*27.5*	9.3				0	0	4	8
3.5	*11.8*	6.0	8.0	*7.4*		0		48	7
21.6	*75.1*	43.4	6.5	*6.0*		1+	trace	72	4
4.5	*15.4*	8.5					0		3
10.2	*34.5*	18.8	9.1	*8.9*		0	0	82	5
15.5	*51.6*	19.7				1+	trace	90	25
2.4	*8.0*	3.2							9
0.8	*2.4*								40
9.1	*30.3*	9.7				trace	0	58	
2.2	*7.5*	2.0				0		66	
65.7	*219.6*	103.5	22.5	*22.4*	5.1	trace		66	8
7.8	*26.2*	13.3	11.3	*11.1*	8.5	1+	1+	42	12
4.0	*13.4*	7.3							25
12.9	*43.1*	30.9	17.5	*17.0*		1+		30	8
1.4	*4.8*		7.1	*4.4*		0		10	30
12.7	*42.4*	28.1	11.5	*11.2*	11.3	1+	0	32	19
0.6	*2.7*	2.0							11
0.9	*3.1*	1.2	5.5	*3.1*		0		0	7
1.6	*5.4*		6.0	*4.1*		0	0	0	7
11.1	*37.7*	21.6	17.2	*17.0*	16.4	2+	2+	28	53
7.0	*23.5*		13.9	*13.8*		0	0	0	43

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum Iron (μg %)	TIBC (μg %)
75 J S	F 69	A. of Inf.[1]	Chronic cholecystitis and infiltration of the liver with round cells and histocytes	8.5	200	256
76 S G	M 29	Rec. hem.[2]	Gastric ulcer Old and recent history of hemorrhage	14 7		
77 G L	40	"	Gastric ulcer Old and recent history of hemorrhage	17 0		
78 J H	36	"	Gastric ulcer Blood donor until several months ago	17.0		
79 S J	38	"	Gastric ulcer. Recent history of hemorrhage	15.2	91	457
80 A K	53	"	Gastric ulcer. History of hemorrhage three months ago	14 7	160	360
81 L S	22	"	Gastric ulcer Recent hemorrhage	16.2	119	314
82 B G	40	"	"	14 4	199	262
83 O G	38	"	Gastric ulcer Recent hemorrhage (one month ago)	13.8	144	357
84 S M	Fm 42	"	Gastric ulcer. Past and recent hemorr			
85 H S	50	"	Uncomplicated cholelithiasis. Myoma uteri and menorrhagia	12.1	76	430
86 A L	43	"	Chronic cholecystitis. Myoma uteri and menorrhagia	12.2		
87 N S	M 37	Past hem.[3]	Gastric ulcer Past history of hemorrhage	14 1		
88 S S	60	Rec. hem.	Gastric ulcer Past and recent history of hemorrhage	13.5		
89 B M	32	B + old hem.[4]	Gastric ulcer Old history of hemorrhage (one year go)	13.6	88	218
90 B N	45	"	Gastric ulcer Old history of hemorrhage (one year ago)	14 0	90	314
91 J H	44	"	Post-gastrectomy Old history of hemorrhage (4 years ago)	13 6	175	403
92 A J	51	"	Old history of hemorrhage (one year go)	14 7	181	305
93 A K	F 70	"	Cholelithiasis. Prolapsus uteri operata with old history of hemorrhage	12.4	216	411
94 B K	52	"	Uncomplicated cholecystopathy Old history of hemorrhage. Hernia diaphragmatica	11.5	100	466
95 J R	M 40	Fe def. a.[5]	Gastric ulcer with massive hemorrhage old and recent. Iron deficiency anemia	11 6		
96 H P	50		Gastric ulcer with old and recent hemorrhage. Iron deficiency anemia	12.0	121	476
96b S V	52		Polya gastr (12 years ago). Past and recent hemorrhage. Iron deficiency anemia	11 9	86	316
97 B K	57	Inf + Rec. hem.	Cancer pancreatis. Post hemorrhagic (recent) anemia	9.5	129	281
98 O K	72	"	Gastric ulcer Recent hemorrhage. Tbc pulm.	12.9	47	2 8

[1] Anemia of infection or toxicity [2] Recent hemorrhage, (within 3 months before study) [3] Past hemorrhage (more than 3 months ago) Old hemorrhage (at least one year before study)
[5] Iron deficiency anemia Fm = Menstruating women

Non-haemin iron in surgical liver biopsy specimens			Non-haemin iron in bone marrow aspirates						
			Chemical determination (μg Fe/mg DNA)			Stainable iron			
Total non-haemin iron (mg Fe/100 g liver)		Ferritin iron (mg/100 g)	Total non-haem iron						
					Ferritin			Sideroblasts	ESR
wet wt	dry wt	dry wt	uncorr.	corr.	corr.	Smears	Sections	(%)	mm/h
13.8	46.8	33.3				1+	2+	50	58
3.7	12 4						0	32	2
23.0	6.7	26.3				1+	1+	60	3
5.1	17.0	0 6				1+	0	74	2
8.2	27.5	9.5				0	0	4	8
3.5	11.8	6.0	8 0	7 4		0		48	7
21.6	75 1	43.4	6 5	6.0		1+	trace	72	4
4.5	15 4	8 5					0		3
10.2	34.5	18 8	9 1	8.3		0	0	82	5
15.5	51.6	19 7				1+	trace	90	25
2.4	8 0	3.2							9
0.8	2 4								40
9 1	30.3	9 7				trace	0	58	
2.2	7.5	2.0				0		66	
65.7	219 6	103 5	22.5	22.4	5.1	trace		66	8
7 8	26 2	13.3	11.3	11 1	8.5	1+	1+	42	12
4.0	13 4	7.3							25
12 9	43 1	30 9	17.5	17.0		1+		30	8
1 4	4.8		7 1	4 4		0		10	30
12 7	42 4	28.1	11.5	11.2	11.2	1+	0	32	19
0 6	2.7	2.8							11
0 9	3 1	1.2	5.5	5 1		0		0	7
1 6	5 4		6.0	4 1		0	0	0	7
11.1	3 .7	21.6	17.2	17.0	16 4	2+	2+	28	53
7.0	23.3		13.9	13.8		0	0	8	43

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum iron (μg %)	TIBC (μg %)
99 HK	M 63	Inf + Rec. hem.	Carcinoma coli. Recent hemorrhage	11 7	62	329
100 VE	55	Fe def a.[1]	Gastric ulcer Past history of hemorrhage Iron deficiency anemia	12.4		
101 NT	38	Old hem.	Gastric ulcer. Old history of hemorrhage (3 years ago)	13 0	179	372
102 JA	58	Fe def a.	Gastric ulcer Past history of hemorrhage. Iron deficiency anemia	13.0		
103 PF	40	Past hem. + Polya[2]	Polya gastrectomy one year ago. Past history of hemorrhage.	12.1		
104 HA	50	Fe def. a.	Polya gastrectomy one year ago. Old and recent history of hemorrhage Iron deficiency anemia. Cholecystopathy	11 6		
105 SS	48	A. of inf.[3]	Polya gastrectomy four years ago. Old history of hemorrhage Anemia of infection	13 2	86	232
106 HL	48	Polya	Polya gastrectomy 13 years ago. Uncomplicated cholelithiasis. No history of hemorrhage	15 1		
107 LV	60	Polya + inf	Polya gastrectomy (seven years ago) No history of hemorrhage. Chronic cholecystopathy	12.7	145	421
108 WM	61	B + Polya	Polya gastrectomy (9 years ago). No history of hemorrhage. Cholelithiasis	14 6	288	537
109 KS	44	Inf	Polya gastrectomy (9 years ago). No history of hemorrhage. Chronic cholecystopathy	13 1	60	357
110 VK	65	Inf. + Jaun.[4] Polya	Polya gastrectomy (28 years ago). No history of hemorrhage. Choledocholithiasis and jaundice	13 9		
111 OR	37	B + Polya	Polya gastrectomy (2 years ago). No history of hemorrhage. Cholelithiasis	13 9	148	317
112 GH	43	A. of inf. Polya	Polya gastrectomy (3 years ago). Acute cholecystitis. No history of hemorrhage. Anemia of infection	11 9		
113 SS	74		Polya gastrectomy (11 years ago). No history of hemorrhage. Gangrenous cholecystitis. Anemia of infection.	11 1	40	275
114 PT	47	Bill I[5]	Billroth I gastrectomy (2 years ago). No history of hemorrhage. Chronic cholecystitis and acute pancreatitis	15 1		
115 ZK	56	B	Billroth I gastrectomy (20 years ago). No history of hemorrhage. Cholelithiasis	14.2	198	300
116 SE	53	Bill I	Billroth I gastrectomy (13 years ago). No history of hemorrhage. Cholelithiasis	14.2	114	305
117 MJ	63	Bill I	Billroth I gastrectomy (22 years ago). No history of hemorrhage. Cholelithiasis	12.8		
118 LF	52		Callous penetrating gastric ulcer	14.5		

[1] Iron deficiency anemia [2] Previously undergone partial gastrectomy according to the Polya technic [3] Anemia of infection or toxicity [4] Jaundice [5] Previously undergone partial gastrectomy according to the Billroth I technic

Non-hemin iron in surgical liver biopsy specimens			N h mi i ln b s max w pl t s — Chemical determination (μg Fe/mg DNA)			Stainable iron			
Total non-hemin iron (mg Fe/100 g liver)		Ferritin iron (mg/100 g)	Total non-hem iron					Sidero-blasts	ESR
wet wt	dry wt	dry wt	uncorr.	corr	Ferritin corr.	Smears	Sections	(%)	mm/h
2.2	7.2	5.0	9.8	9.5	9.9	0	trace	16	64
3.0	10.0					0	0	4	
7.4	24.6	11.6	9.3	8.4		0	0	72	5
2.6	8.7	3.0				trace	0	21	
3.9	12.6	5.7				1+		44	5
1.0	[illegible]	1.8				0	0		8
9.8	32.0	19.8							7
2.8	9.7								18
2.3	7.6	4.3	8.3	8.3	7.2	0	0	60	20
1.2	4.0	1.9	12.7	6.9		0		70	6
3.4	11.5	6.2	9.6	9.2		1+	0	36	10
3.9	12.1	6.3							
4.6	22.3		18.6	15.3		1+		22	8
9.8	33.6	21.7							26
21.7	7.5	41.0	24.4	25.2	18.6	2+	2+	32	13
14.5	48.5	29.5							13
11.4	38.9	28.1					0	53	18
23.2	75.4	55.3	9.7	8.5		1+		88	4
8.8	18.8 29.3	14.2					1+	20	13 7

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum Iron (μg %)	TIBC (μg %)
99 H K	M 63	Inf. + Rec. hem.	Carcinoma coli. Recent hemorrhage	11 7	62	*329*
100 V E	55	Fe def. [1]	Gastric ulcer Past history of hemorrhage. Iron deficiency anemia	12.4		
101 N T	38	Old hem.	Gastric ulcer Old history of hemorrhage (3 years ago)	13.0	179	*372*
102 J A	58	Fe def a.	Gastric ulcer Past history of hemorrhage. Iron deficiency anemia	13 0		
103 P F	40	Past hem. + Polya[2]	Polya gastrectomy one year ago. Past history of hemorrhage	12.1		
104 H A	50	Fe def. a	Polya gastrectomy one year ago. Old and recent history of hemorrhag Iron deficiency anemia. Cholecystopathy	11 6		
105 S S	48	A. of inf [3]	Polya gastrectomy four years ago. Old history of hemorrhage. Anemia of infection	13.2	86	*232*
106 H L	48	Polya	Polya gastrectomy 13 years ago. Uncomplicated cholelithiasis. No history of hemorrhage	15 1		
107 L V	60	Polya + inf.	Polya gastrectomy (seven years ago.) No history of hemorrhage. Chronic cholecystopathy	12.7	145	*421*
108 W M	61	B + Polya	Polya gastrectomy (9 years ago) No history of hemorrhage. Cholelithiasis	14 6	288	*53*
109 K S	44	Inf	Polya gastrectomy (9 years ago). No history of hemorrhage. Chronic cholecystopathy	13.1	80	*55*
110 V K	65	Inf + Jaun.[4] Polya	Polya gastrectomy (28 years ago). No history of hemorrhage. Choledocholithiasis and jaundice	13.9		
111 O R	37	B + Polya	Polya gastrectomy (2 years go). No history of hemorrhage. Cholelithiasis	13.9	148	*31*
112 G H	43	A. of inf. Polya	Polya gastrectomy (5 years ago). Acute cholecystitis. No history of hemorrhage. Anemia of infection	11 9		
113 S S	74		Polya gastrectomy (11 years ago). No history of hemorrhage Gangrenous cholecystitis. Anemia of infection.	11 1	40	*275*
114 P T	47	Bill I[5]	Billroth I gastrectomy (2 years ago). No history of hemorrhage. Chronic cholecystitis and acute pancreatitis	15.1		
115 Z K	56	B	Billroth I gastrectomy (20 years ago). No history of hemorrhage. Cholelithiasis	14.2	198	*300*
116 S E	53	Bill I	Billroth I gastrectomy (13 years ago) No history of hemorrhage. Cholelithiasis	14.2	114	*305*
117 M J	63	Bill I	Billroth I gastrectomy (22 years ago). No history of hemorrhage. Cholelithiasis	12.8		
118 L F	52		Callous penetrating gastric ulcer	14.5		

[1] Iron deficiency anemia [2] Previously undergone partial gastrectomy according to th Polya technic [3] Anemia of infection or toxicity Jaundice [5] Previously undergone partial gastrectomy according to the Billroth I technic

Non-hemin iron in surgical liver biopsy specimens			N h mln l l b m w pl t Chemical determination (μg F/mg DNA)			Stainable iron			
Total non-hemin iron (mg Fe/100 g liver)		Ferritin iron (mg/100 g)	Total non-hem iron						
wet wt	dry wt	dry wt	uncorr	corr	Ferritin corr	Smears	Sections	Sideroblasts (%)	ESR mm/h
1.5	5.2	2.7				trace	0	6	
13.3	44.3	31.0	9.8	8.5		0	0	62	4
2.1	7.4	4.6							3
6.4	22.0	15.0	21.4	20.1		1+		47	6
28.6	111.9	63.9				2+		50	
11.5	32.4	17.8	18.2	18.2		1+	1+	40	7
7.1	24.2	20.1	19.7	19.2		1+	0	56	4
3.2	10.9	3.3	14.1	7.3					4
13.2	44.1	23.9	13.4	12.3		2+	1+	58	5
24.2	88.9	71.3					1+		16
10.9	36.8	18.4							14
16.1	58.6	39.1	20.2	18.8		trace		22	8
23.4	78.0	43.3					trace	48	18
20.4	68.2	50.7		33.3	28.0	3+		64	54
7.3	29.1	20.9		9.3		0		52	7
12.6	48.2	27.0							10
1.6	5.3	2.3		5.2		0	0	58	5
3.9	13.3	6.5				0		0	35
11.5	38.2	24.0		8.3		0	0	60	4
4.5	15.0	6.3	5.4	4.4	3.0	0	0	12	8

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum iron (µg %)	TIBC (µg %)
119 A A	M 61	B[1]	Gastric ulcer and pyloric stenosis	14.8	59	380
120 J R	44	"		18.0	143	287
121 K A	23	"	Hypertrophic gastritis. No history of hemorrhage	16.7	178	296
122 S C	34	"	Polya gastrectomy (2 years ago). Uncomplicated cholelithiasis. Got oral iron therapy during several months	14.5	184	336
123 B F	63		Hemorrhoids with occasional small bleedings. Gastric ulcer. Oral iron therapy	13.7		
124 N T	61	"	Gastric ulcer. Abuse of salicylates. No history of hemorrhage	14.7	207	56
125 E A	50	Inf.[2] + Past. hem.[3]	Past history of hemorrhage. Steatosis hepatis alcoholica and moderate portal fibrosis. No change in structure. Gastric ulcer	14.7	185	328
126 R A	52		Cancer ventriculi	14.0	147	363
127 B E	50	Inf	Cancer ventriculi. Gastric ulcer. Cholecystopathy	15.2	158	268
128 E K	F 52	B	Uncomplicated cholelithiasis. Oral iron therapy	11.6	118	355
129 O A	54		Uncomplicated cholelithiasis. Prolonged oral iron therapy	11.9		
130 B M	Fm 46	B	Uncomplicated cholelithiasis. Prolonged oral iron therapy	12.0	173	250
131 K E	44	Inf.	Callous penetrating gastric ulcer. Hepatitis chronica levis (histol. diagnosis). Oral iron therapy	13.8		
132 O R	F 57	Inf + Jaun.[4]	Chronic cholecystitis and jaundice	11.7	1.3	4 5
133 A E	62	B + Polya	Polya gastrectomy (3 years ago). Cholelithiasis. No history of hemorrhage	14.7	186	323
134 K H	48	B	Chronic cholecystitis. Fistula and operation (one year ago)	11.9		
135 E I	48		Callous gastric ulcer. Anorexia nervosa	12.8	142	2 9
136 A G	Fm 44	"	Recurrent gastric ulcer. Chronic abuse of alcohol	11.9	80	383
137 M K	42	"	Mitralstenosis. Uncomplicated cholelithiasis	12.4		
138 T A	18	"	Polyposis coli transversi	11.7	128	415

[1] Basal subject [2] Infectious or toxic state without anemia [3] Past hemorrhage (more than 3 months ago)

[4] Jaundice Fm = *Menstruating women*

N h ml l l bo m w pl t

Non-hemin iron in surgical liver biopsy specimens			Chemical determination (*μg Fe/mg DNA*)			Stainable iron			
Total non-hemin iron (*mg Fe/100 g liver*)		Ferritin iron (*mg/100 g*)	Total non-hem iron		Ferritin			Sidero-blasts	ESR
wet wt	dry wt	dry wt	uncorr.	corr.	corr	Smears	Sections	(%)	mm/h
1.5	*5.2*	2.7				trace	0	6	
13 3	*44.5*	31.0	9.8	*8.5*		0	0	62	4
2.1	*7 4*	4 6							3
6.4	*22.0*	15.0	21 4	*20.1*		1+		47	6
28.6	*111.9*	63.9				2+		50	
11.5	*32.4*	17.8	18.2	*18.2*		1+	1+	40	7
7 1	*24.2*	20.1	19 7	*19.2*		1+	0	36	4
3 2	*10.9*	3.3	14 1	*7.3*					4
13.2	*44 1*	23.9	13.4	*12.3*		2+	1+	38	5
26.2	*88.9*	71.3					1+		16
10 9	*56.8*	18.4							14
16.1	*58.6*	39 1	20.2	*18.8*		trace		22	8
23 4	*78.0*	43.3					trace	48	18
20.4	*68.2*	50.7		*35.3*	28 0	2+		64	54
7.3	*29 1*	20.9		*9.5*		0		52	7
12.6	*38.2*	27.0							10
1.6	*5.3*	2.3		*5.2*		0	0	38	5
3.9	*13 3*	6 5				0		0	35
11.5	*38.*[illegible]	24 0		*8.3*		0	0	60	4
4.5	*15.0*	6.3	5.4	*4 4*	3 0	0	0	12	8

Subject		Sex and age		Group	Clinical diagnosis	Hb (g %)	Serum Iron (µg%)	TIBC (µg%)
139	S J	M	30	B[1] + C[2]	Hypercholesterolemia	16.1	147	395
140	B B		46	„	Uncompl. gastric ulcer. No hemorrhage	15.6	186	289
141	H B		23	„	„	16.2	192	323
142	L A		61	„	Uncomplicated cholelithiasis	13.6	229	267
143	E J		50	„	Thyreotoxicosis in remission	14.3	108	333
144	L G	Fm	23	„	Healthy volunteer (nurse)	13 4	118	331
145	A L		23	„	„	14 0	160	390
146	J K		22	„	„	12.7	143	325
147	A S		50	„	Cicatrix duodeni. No hemorrhage	11 6	131	366
148	Ö A	F	46	„	Uncomplicated gastric ulcer No hemorrhage. Menopaus 5 months ago Oral iron therapy for short time	11 7	176	368
149	L S	F	63	„	Uncomplicated cholelithiasis	13.3	142	283
150	S G	M	50	Fe def. a.[3]	Polya gastrectomy Chronic glomerulonephritis. Iron deficiency anemia	10.5	32	387
151	B V		26	„	Morbus Bechterew Iron deficiency anemia	10.4	49	400
152	E E		70		Post Polya gastrectomy Iron deficiency anemia	9 8	43	420
153	L A		68	„	Chronic gastric ulcer and past hemorrhage Iron deficiency anemia	8.3	14	351
154	Lj K		42		Post-Polya gastrectomy Iron deficiency anemia.	11 0	50	354
155	A K		62		Chronic hemorrhage. Hemorrhoides. Iron deficiency anemia.	10.3	21	445
156	N G		54		Iron deficiency anemia. Coilonychia.	12.6	45	331
157	K E		63		Post Polya gastrectomy Iron deficiency anemia.	10.7	28	466
158	Ö E		58	„	Post-Polya gastrectomy Cancer prostatae. Iron deficiency anemia.	9 4	30	336
159	T S		55		Sclerosis disseminata. Iron deficiency anemia (occult hemorrhage)	9 8		
160	O H		41		Post Polya gastrectomy Iron deficiency anemia.	11 8	37	391
161	S K		50		Post Polya gastrectomy Iron deficiency anemia.	11 1	53	[illegible]
162	H T		45		Post Polya gastrectomy Iron deficiency anemia.	9 5	40	427
163	S E		43		Post Polya gastrectomy Iron deficiency anemia.	10.9	30	368
164	S V		52		Post-Polya gastrectomy Iron deficiency anemia.	11 9	86	346
165	S G		52		Post Polya gastrectomy Iron deficiency anemia.	11 1	46	453
166	B A		45		Post Polya gastrectomy Iron deficiency anemia.	11 1	52	409
167	G B	Fm	16		Iron deficiency anemia. Pyelonephritis.	9.2	53	625
168	B B		25	„	Iron deficiency anemia.	9 9	41	336

[1] Basal subject [2] Control subject [3] Iron deficiency anemia Fm = Menstruating women

N hemi i l b m w pl t

Chemical determination (μg Fe/mg DNA)			Stainable iron			
Total non-hem Fe		Ferritin Fe			Sideroblasts	ESR
uncorr.	corr.	corr.	Smears	Sections	%	mm/h
14.8	14.6		3+	0	78	7
29 6	27.9		1+		60	2
11.7	9 4		0		40	2
24 7	21.7		trace	2+	29	11
26.2	25.6		3+	2+	78	10
5 8	4.6			0	8	
6.2	4.3				38	
8.1	7.0		0	trace	42	
8.1	6.3		trace		22	9
10.8	8.2		1+	trace	66	5
17 7	17 4		3+		66	22
1 9	1 7		0	0	0	
3.3	2 7		0		8	20
5.4		4 9	0		4	
1 7	1.8		0	0	12	17
8.1	7 7		0		0	
1.3	1 1		trace	trace	6	25
2.9	2 9		0	0	2	
4.6	4 6			0		20
3.4	3 3	2.6	0	0	5	35
1.5	1 5		0		0	
1 8	1 7		0	0	2	5
4.0	3 1		0	0	1	5
1 1	0 0		0	0	0	
2 1	1 7		0	0	2	
6.0	4 1		0	0	0	7
1	1 3		0	trace	0	
1 2	1 1		0	0	0	
3 6			0	0	0	
5 3			0		0	28

Subject		Sex and age		Group	Clinical diagnosis	Hb (g %)	Serum Iron (µg%)	TIBC (µg%)
139	S J	M	30	B[1] + C[2]	Hypercholesterolemia	16.1	147	395
140	B B		46	..	Uncompl. gastric ulcer No hemorrhage	15.6	186	289
141	H B		23	..	..	16.2	192	323
142	L A		61	..	Uncomplicated cholelithiasis	13.6	229	267
143	E J		50	..	Thyreotoxicosis in remission	14.3	108	333
144	L G	Fm	23	..	Healthy volunteer (nurse)	13.4	118	331
145	A L		23	..	..	14 0	160	390
146	J K		22	..	..	12.7	143	325
147	A S		50	..	Cicatrix duodeni. No hemorrhage	11 6	131	366
148	Ö A	F	46	..	Uncomplicated gastric ulcer No hemorrhage. Menopaus 5 months ago. Oral iron therapy for short time	11 7	176	368
149	L S	F	63	..	Uncomplicated cholelithiasis	13.3	142	283
150	S G	M	50	Fe def a.[3]	Polya gastrectomy Chronic glomerulonephritis. Iron deficiency anemia	10.5	32	387
151	B V		26		Morbus Bechterew Iron deficiency anemia	10.4	49	400
152	E E		70	..	Post Polya gastrectomy Iron deficiency anemia	9 8	43	420
153	L A		68	..	Chronic gastric ulcer and past hemorrhage Iron deficiency anemia	8.3	14	351
154	Lj K		42		Post Polya gastrectomy Iron deficiency anemia.	11 0	50	334
155	A K		62	..	Chronic hemorrhage Hemorrhoides. Iron deficiency anemia.	10.3	21	445
156	N G		54	..	Iron deficiency anemia. Coilonychia	12.6	45	337
157	K E		63	..	Post Polya gastrectomy Iron deficiency anemia.	10.7	28	466
158	Ö E		58		Post Polya gastrectomy Cancer prostatae Iron deficiency anemia.	9 4	30	316
159	T S		55		Sclerosis disseminata. Iron deficiency anemia (occult hemorrhage)	9 8		
160	O H		41	..	Post Polya gastrectomy Iron deficiency anemia.	11 8	37	391
161	S K		50		Post Polya gastrectomy Iron deficiency anemia.	11 1	53	3 8
162	H T		45		Post Polya gastrectomy Iron deficiency anemia.	9 5	40	42
163	S E		43	..	Post Polya gastrectomy Iron deficiency anemia.	10.9	30	369
164	S V		52		Post Polya gastrectomy Iron deficiency anemia.	11 9	86	316
165	S G		52		Post Polya gastrectomy Iron deficiency anemia.	11 1	46	453
166	B A		45		Post-Polya gastrectomy Iron deficiency anemia	11 1	52	409
167	G B	Fm	16		Iron deficiency anemia. Pyelonephritis.	9.2	53	625
168	B B		25	..	Iron deficiency anemia.	9.9	41	316

[1] Basal subject [2] Control subject [3] Iron deficiency anemia Fm = Menstruating women

N b ml i j bo m w pl t

Chemical determination (µg Fe/mg DNA) Total non-hem Fe uncorr.	corr.	Stainable iron Smears	Sections	Sideroblasts %	ESR mm/h
2.7	2.5	0		0	46
0.8	0.8	0	0	2	41
3.4	3.4		0		50
4.4	3.4				
5.6	5.6				
4.9	4.4				
6.4	6.1	0	0	20	
5.9	5.4	0		24	
2.5	2.5	0	0	10	
4.1	3.7	0	0	54	
4.2	3.7	0	0	2	
6.3	3.2				
10.1	6.3	0		48	
4.6	4.1	trace	0	50	
9.1	8.2	0	0	48	
4.7	3.6	trace		24	
6.9	6.5	0	0	64	
3.1	2.6	0	0	64	
11.7	6.6				
3.4	2.7	trace		40	
2.9	2.5	0		24	
5.6	5.0	0	0	22	
4.7	3.7	trace		32	
5.0	3.7	0		8	
10.7	10.1	0	0	14	
4.4	4.0	0	0	55	
8.5	8.6	0	0	20	
4.0	3.0				
9.6	9.3	0		32	
3.7	2.7				
11.3	10.4	1+	0	70	
10.2	7.0	0	0	8	
4.0	3.3	0		27	
14.7	1 7			40	
3.3	2.7	0	0	8	
28.2	23.1	trace	1+	60	
4.2	3.4	0	0	34	
19.5	19.4	1+	trace	32	
11.0	8.9			40	
8.0	6.9	0		32	

Subject		Sex and age	Group	Clinical diagnosis	Hb (g%)	Serum Iron (μg%)	TIBC (μg%)
169	L A	Fm 45	Fe def a.[1]	Infarctus myocardii. Diabetes mellitus. Iron deficiency anemia.	8.9	29	368
170	N M	F 82	"	Polypus ventriculi. Iron deficiency anemia.	5.9	17	337
171	R E	59	"	Rheumatoid arthritis. Mitralstenosis Splenomegaly Iron deficiency anemia.	9.5	45	430
172	G K	M	B. d.[2]	Blood donor	14.3	83	403
173	J H		"	"	14.0	60	470
174	Å L		"	"	13.8	53	433
175	G A		"	"	14.6	102	342
176	H K		"		14.7	111	433
177	C H			"	13.7	71	458
178	A S		"	"	15.2	121	448
179	A Å		"	"	14.8	101	444
180	St L			"	14.2	137	403
181	B G		"		14.2	145	384
182	B A		"	"	13.9	92	307
183	L G		"		13.6	176	316
184	J S		"	"	15.0	141	397
185	O K		"		14.3	84	342
186	G K			"	14.6	87	323
187	J L		"		15.2	226	293
188	L S		"	"	14.2	117	490
189	L S		"	"	14.2	93	344
190	N S		"		13.8	77	401
191	S N		"	"	14.0	132	385
192	E L		"		14.0	149	378
193	B F				13.6	78	311
194	A C			"	15.0	72	430
195	E L		"	"	13.8	279	417
196	F E				14.5	127	504
197	L R		"	"	15.2	80	451
198	O E		"		13.9	139	336
199	E V		B[3]	Post Polya gastrectomy No hemorrhage	13.7	89	439
200	E E		"	"	14.1	111	470
201	A L				13.9	140	400
202	A E		"	"	13.8	198	345
203	P K		"		16.0	132	418
204	R H		"	"	14.5	114	317
205	B E		"		13.9	147	299
206	K G				14.0	150	278
207	P S		"		14.3	109	293
208	C A		"	"	14.3	167	366

[1] Iron deficiency anemia [2] Blood donor [3] Basal subject Fm = Menstruating women

N b ml l n l b m w pl t

Chemical determination (μg Fe/mg DNA)			Stainable iron			
Total non-haem Fe		Ferritin Fe			Sideroblasts	ESR
uncorr.	corr.	corr.	Smears	Sections	%	mm/h
11.2	9 4		0	0	50	
7 4	7.0		0	trace	50	
14.6	13.8		2+	1+	76	
6.6	4.5		trace		40	
19.5	19 1		2+		68	
10.3	9.7		0		44	
9.6	9.6		trace			
6.4	5.6		trace		30	
28.9	27.0		trace			
11.0	6.9		0		26	
4.9	4.2		0	0	18	
9.0	8.7		0	0	28	
3.6	3.3		0		50	
17 0	15.5		0		34	
32.6	31.5			0		
23.3	23 1		1+	0	63	
17.9	17.8		0	1+	35	
10.1	9.6		0	trace	30	
13 3	11.0		2+	1+	72	
10.0	5.8		0	trace	28	
8.7	7.8		0		36	
20 0	18.9		trace	1+	34	
18 6	15 3		1+		22	
12.5	7.9			trace		
7.2	6 5		trace	trace	48	
25 6	24.6		2+	1+	44	
2.7	1 4		0	trace	10	
9 7	9 1		0		54	
5 6	4 3		trace		64	
12 7	6.9		0		70	
8 6	7 3			trace		
6 6	5 1		0	0	28	
4.6	2 9		0	0	74	
13 2	14 8		3+		76	
8.6	7 9		trace	3+	64	
2 5	1 9		0	0	60	
13 9	3 8					
12 3	12 2	9.8	trace	trace	4	
	1 9		0	0	6	12
4 0			0		0	25

Subject		Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum iron (μg%)	TIBC (μg%)
209	H B	M	B[1]	Post Polya gastrectomy No hemorrhage	13.9	211	299
210	D B		"	"	14 6	164	268
211	K B		"	"	14 6	210	329
212	B K		"		15.2	214	320
213	N O		"		15.7	129	293
214	StjH		"		14.3	129	400
215	R Å		"	"	14.3	115	345
216	S S		"		14.3	117	332
217	S S		"	"	13 8	132	313
218	F Å		"	"	13 6	167	2 5
219	B S		"		14.5	147	330
220	L K		"		14.5	116	304
221	A K		"	"	14.2	58	366
222	N E		"	"	15 4	150	399
223	O O		"		14.5	177	258
224	B J				14 4	128	204
225	M G		"		13.9	75	304
226	J K		"	"	14.2	67	322
227	J K		"		13 9	87	338
228	L B		"		13.9	81	378
229	L E		"	"	13.7	154	415
230	J G				15 4	149	342
231	R O			"	13.9	148	317
232	A B				14 7	176	34
233	P J		"	"	13 9	143	268
234	L H		"		13.7	155	290
235	N B		"		14 5	114	415
236	E S		"		14.3	117	317
237	J H				14.2	150	307
238	V M		"		14 6	288	53
239	J I				13.6	140	315
240	J T				14 6	111	372
241	K V				13.9	146	390
242	L O				13 6	135	268
243	L I				14.3	86	280
244	P O				15.5	113	4 5
245	O K	72	Rec. hem.[2]	Gastric ulcer and hemorrhage	12.9	47	2 8
246	G N	42		Chronic gastric ulcer. Recent and old hemorrhage.	9 1	48	336
247	B K	45		Gastric ulcer and recent hemorrhage Hemolytic anemia.	9 7	67	362
248	N J	61		Hemangiomas of the intestine and recent hemorrhage.	8.5		

[1] Basal subject [2] Recent hemorrhage

N heml l l bo m w pl t

Chemical determination (μg Fe/mg DNA)			Stainable Iron			
Total non-hem Fe		Ferritin Fe			Sideroblasts	ESR
uncorr.	corr.	corr.	Smears	Sections	%	mm/h
8.8	8.5		0	0	0	
21.1	21.0	17.0	1+	2	12	145
20.7			1+	1+	90	2
11.0	6.0		0			2
12.2			0	0	72	10
4.0	2.9		0		0	
6.4	5.8		trace	0	38	
12.0	13.4		0	1+	12	10
14.3	14.3	8.4	2+	0	94	39
8.0			0		0	19
52.0	52.7	49.7	2+		12	22
22.5	22.4		2+	1+	48	20
17.8	17.8		1+		69	29
21.7	21.6	16.5	2+	2+	26	45
11.4			0	trace	94	
11.5	11.5		1+	2+	29	90
13.1	13.1		1+	1+	4	70
41.7	41.8	34.3	1+	3+	36	35
42.7	42.9	27.9	3+	1+	86	5
15.4	15.3		1+		46	69
33.4	33.4	27.3	1+	1+	60	16
58.3	59.3	46.6	3+	3+	82	75
74.6	74.6		3+	2+	70	36
84.6	84.6	22.6	3+ – 4+	3+	96	37–70
7.3	7.3		3+		60	30

Subject		Sex and age		Group	Clinical diagnosis	Hb (g %)	Serum iron (μg%)	TIBC (μg%)
249	G Å	M	35	Rec. hem.[1]	Blood donor. Hydronephrosis. Recent and old hemorrhage.	11 9	44	343
250	N K		50	Inf.[2] + Rec. hem.	Intestinal hemorrhage. Chronic pyelonephritis and uremia. Anemia.	6.7	93	216
251	A K		43	Rec. hem.	Gastric ulcer and recent hemorrhage	14 4		
252	A S		47	B[3] + old hem.	Hemorroidectomy 10 years ago. Old history of hemorrhage.	16.3	205	308
253	V G		61	Old hem.	Gastric ulcer Old hemorrhage.	17.8	243	335
254	K M	F	51	Rec. hem.	Gastric ulcer Recent and old hemorrhage Pancreatic adenoma.	10.3	53	357
255	H G	Fm	24	"	Menorragia. Recent and old hemorrhage.	10.3	74	250
256	C I		50	"	Gastric ulcer and recent hemorrhage.	8.7	50	287
257	J H	F	67		Gastric ulcer Occasional hemorrhages from hemorrhoids.	11 9	58	241
257b	J M	Fm	48	Rec. hem. + Inf.	Menorrhagia. Hemoptysis. (obscure pulmonary disease)	11.9	63	397
258	I V	M	27	Inf.	Regional ileitis.	13.8	91	359
259	J S		65	Inf + Jaun.[4]	Chronic cholecystopathy and jaundice.	14 7	132	
260	A K		70		Active alcoholic liver cirrhosis and jaundice	14 6	222	470
261	A O		35	Inf	Bronchopneumonia and venous thrombosis.	13.7	103	229
262	N A		80		Uremia.			
263	H R		50	A. of inf.[5]	Active alcoholic liver cirrhosis. Probably former hemorrhage.	5.4	21	341
264	P J		80		Stomatitis ulcerosa. Fever	10.7	44	195
265	R A		75	"	Carcinoma coli. Got blood transfusions.	11.3	81	259
266	O F		43	A. of inf + transf.	Chronic and active liver cirrhosis. Chronic pancreatitis. Several years ago got about 30 blood transfusions. (scarce hemorrhage).	9.3	45	200
267	D E	Fm	47	Inf. + J un.	Chronic and acute cholecystitis with jaundice.	14 6	71	290
268	S E	F	73	Inf + transf.	Diverticulitis sigmoidei. Transversostomia. Earlier got 8 units of blood.	12.7		
269	A A		53	Inf + exog. Fe load.	Rheumatoid arthritis. Earlier got 1000 mg colloidal iron i.m. and oral iron therapy	11.6	81	235
270	N H		65		Diabetes mellitus. Anemia perniciosa in remission. Earlier got 1500 mg colloidal iron parenteraly	12.2	89	265
271	L K		56	A. of inf + exog. Fe load.	Chronic alcoholic intoxication. Malnutrition. Hypoproteinemia. Pyridoxin deficiency Earlier got oral iron therapy during 3—4 months.	5.1	198	212
272	S T		47	A. of inf.	Active liver cirrhosis. Malnutrition. Abuse of alcohol.	9 9	90	221

[1] Recent hemorrhage [2] Infectious or toxic state [3] Basal subject [4] Jaundice

[5] Anemia of infection and toxicity Fm = Menstruating women

Old hemorrhage (at least one year before study)

Chemical determination (μg Fe/mg DNA)			Stainable iron			
Total non-haem Fe		Ferritin F			Sideroblasts	ESR
uncorr.	corr.	corr.	Smears	Sections	%	mm/h
8.8	8.5		0	0	0	
21.1	21.0	17.0	1+	2	12	145
20.7			1+	1+	90	2
11.0	6.0		0			2
12.2			0	0	72	10
4.8	2.9		0		0	
6.4	5.8		trace	0	38	
12.0	13.4		0	1+	12	10
14.3	14.3	8.4	2+	0	94	39
8.0			0		0	19
52.0	52.7	49.7	2+		12	22
22.5	22.4		2+	1+	48	20
17.8	17.8		1+		69	29
21.7	21.6	16.5	2+	2+	26	45
33.4			0	trace	94	
11.5	11.5		1+	2+	29	90
13.1	13.1		1+	1+	4	78
41.7	41.8	34.3	1+	3+	36	35
42.7	42.9	27.9	3+	1+	86	5
13.4	13.3		1+		46	69
33.4	33.4	27.3	1+	1+	60	16
58.3	59.3	46.6	3+	3+	82	75
74.6	4.6		3+	2+	70	36
84.6	84.6	22.6	3+ – 4+	3+	96	57–70
7.3	7.3		3+		60	30

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum iron (μg%)	TIBC (μg%)
249 G Å	M 35	Rec. hem.[1]	Blood donor Hydronephrosis. Recent and old hemorrhage.	11.9	44	343
250 N K	50	Inf.[2] + Rec. hem.	Intestinal hemorrhage. Chronic pyelonephritis and uremia. Anemia.	6.7	93	236
251 A K	43	Rec. hem.	Gastric ulcer and recent hemorrhage	14 4		
252 A S	47	B[3] + old hem.	Hemorroidectomy 10 years ago. Old history of hemorrhage	16.3	205	308
253 V G	61	Old hem.	Gastric ulcer. Old hemorrhage	17.8	243	335
254 K M	F 51	Rec. hem.	Gastric ulcer. Recent and old hemorrhage. Pancreatic adenoma.	10.3	53	357
255 H G	Fm 24	„	Menorragia. Recent and old hemorrhage.	10.3	74	250
256 C I	50		Gastric ulcer and recent hemorrhage.	6.7	50	287
257a J H	F 67	„	Gastric ulcer Occasional hemorrhages from hemorrhoids.	11.9	58	241
257b J M	Fm 48	Rec. hem. + Inf	Menorrhagia. Hemoptysis. (obscure pulmonary disease)	11 9	63	397
258 I V	M 27	Inf	Regional ileitis.	13.8	91	359
259 J S	65	Inf. + Jaun.[4]	Chronic cholecystopathy and jaundice.	14 7	132	
260 A K	70		Active alcoholic liver cirrhosis and jaundice	14 6	222	420
261 A O	35	Inf	Bronchopneumonia and venous thrombosis.	13.7	103	229
262 N A	80		Uremia.			
263 H R	50	A. of inf.[5]	Active alcoholic liver cirrhosis. Probably former hemorrhage.	5.4	21	341
264 P J	80	„	Stomatitis ulcerosa. Fever	10.7	44	195
265 R A	75		Carcinoma coli. Got blood transfusions.	11.3	81	259
266 O F	43	A. of inf. + transf.	Chronic and active liver cirrhosis. Chronic pancreatitis. Several years ago got about 30 blood transfusions. (scarce hemorrhage).	9.3	45	200
267 D E	Fm 47	Inf. + Jaun.	Chronic and acute cholecystitis with jaundice.	14 6	71	290
268 S E	F 73	Inf + transf.	Diverticulitis sigmoidei. Transversostomia. Earlier got 8 units of blood.	12.7		
269 A A	53	Inf + exog. F load.	Rheumatoid arthritis. Earlier got 1000 mg colloidal iron i.m. and oral iron therapy	11 6	61	235
270 N H	65	„	Diabetes mellitus. Anemia perniciosa in remission.Earlier got 1500 mg colloidal iron parenterally	12.2	89	265
271 L K	56	A. of inf. + exog. Fe load.	Chronic alcoholic intoxication. Malnutrition. Hypoproteinemia. Pyridoxin deficiency Earlier got oral iron therapy during 3–4 months.	5 1	198	212
272 S T	47	A. of inf.	Active liver cirrhosis. Malnutrition. Abuse of alcohol.	9 9	90	221

[1] Recent hemorrhage [2] Infectious or toxic state [3] Basal subject [4] Jaundice
[5] Anemia of infection and toxicity Fm = Menstrus
Old hemorrhage (at least one year before study)

N h ml l l b m ow pl

Chemical determination (µg Fe mg DNA)			Stainable iron			
Total non-hem Fe		Ferritin			Sideroblasts	ESR
uncorr.	corr.	corr	Smears	Sections	%	mm h
25.4	25.3		3+	2+	22	
6.7	6.6	4.9		trace		77
22.4	22.1		2+	2+	72	44
14.2	14.1	8.7	0	1+	38	45
8.2	7.9		2+	2+	2	
21.5	21.3		2+	2+	78	20
66.8	66.8	53.6	3+	3+	100	17
38.0	38.0	11.1	3+		48	40
28.9	28.8	20.2	2+		42	80
27.7	27.7		3+	2+		23
23.1	23.1	17.3	2+		72	15
39.5	39.6	20.0				
53.2	53.3	34.9	3+		62	32
31.3	31.3	21.4	2+	3+	84	10
33.4	33.4		2+	3+	6	51
7.8	7.2		0		20	4
94.9	94.9	65.4	4+	4+	78	40
22.1	22.1		2+	1+	96	36
26.4	26.4	20.6	3+	1+	92	35
20.2	20.2		trace	0	80	29
10.0			0	0	66	
19.3	18.5		1+	0	75	
7.2	4.5		0		82	1
12.7	10.4		1+	1+	20	4
2.9	6		0	0	20	2
1.7	1.6		0	0	0	1
19.2			1+	2+	88	

Subject	Sex and age	Group	Clinical diagnosis	Hb (g %)	Serum Iron (μg%)	TIBC (μg%)
273 A H	F 68	A. of inf.[1]	Sigmoiditis.	8.8	52	270
274 G K	66	A. of inf. + transf.	Chronic pyelonephritis and uremia. Blood transfusions 6 months ago.	10.1	4	32
275 S I	Fm 30	A. of inf. + exog. Fe load.	Chronic pyelonephritis. 1000 mg iron parenterally 6 months ago.	8.8	104	79
276 L R	F 42	„	Sarcoidosis cum hepatosplenomegaly and pancytopenia. Earlier got oral iron and transfusion of one blood unit.	7.0	72	171
277 L M	71	A. of inf.	Rheumatoid arthritis. Oral iron therapy less than one month.	10.1	50	278
278 J G	M 61	Meg. a.[2]	Pernicious anemia.	4.5	233	45
279 A G	76	„	Megaloblastic anemia. Folic acid deficiency	8.2	233	232
280 S F	67	„	Megaloblastic anemia.	5.8	97	240
281 G L	47	„	Pernicious anemia.	4.8	177	255
282 S A	F 76		Megaloblastic anemia. Malabsorption and steatorréa.	7.7	70	270
283 A E	Fm 39	„	Pernicious anemia.	4.5	209	230
284 R H	F 72	„		9.3	51	219
285 L I	64			5.8	284	273
286 E L	M 67	„	Hemolytic anemia. Post splenectomy Tromboeytosis.	12.2		
287 R A	57	Misc. a. + exog. Fe load[3]	Acut. leuchemia. Fever. One months earlier got 1000 mg iron parenterally. Tbc. pulm. sanata.	8.4	48	238
288 L G	55		Chronic lymphatic leuchemia.	14.5	119	397
289 E A	68	Misc. a. + exog. F. load	Refractory anemia and transfusion siderosis	6.9		
290 B A	F 75	A. of inf. + toxicity	Multiple myeloma	7.9	70	
291 P A	82	A. of inf. + toxicity	Acute myeloic leuchemia.	6.3	5	
292 C G	55	A. of inf. + toxicity	Refractory anemia. No transfusion. Oral iron therapy less than one month.	9.6	167	229
293 H O	M 81		Polycytemia vera. Formerly treated with P^{32}	14.8	87	305
294 A G	63		Polycytemia vera. After treatment with busulphan.	15.9		30
295 L G	61		Kyphoscoliosis. Emphysema pulm. Polycytemia secundaria.	19.1	214	370
296 L G	50		Chronic pulmonary embolism. Polycytemia secundaria.	16.9	120	250
297 B L	F 53		Polycytemia vera. After blood letting.	15.9	51	415
298 O H	58		Polycytemia vera.	17.8	50	305
299 H S	68		Polycytemia vera. After treatment with busulphan.	14.0	146	262

[1] Anemia of infection [2] Megaloblastic anemia [3] Miscellaneous anemia and exogenous iron load
Fm = Menstruating women

N b ml l l bo m w plra

Chemical determination (μg Fe/mg DNA) Total non-hem F uncorr.	corr.	Stainable iron Smears	Sections	Sideroblasts %
8 6	7.5		trace	3
29.3	29.[illegible]	2+	2+	90
12 6	12.1	1+		10
3.5	3.5	0	0	2
11.9	10.5	0	0	9

Subject		Sex and ge	Group	Clinical diagnosis	Hb (g %)	Serum Iron (µg%)	TIBC (µg%)
300	J I	M 46	B	Post Polya partial gastrectomy (seven years ago). No hemorrhage.	13.6	140	*305*
301	A G	M 40	"	Post Polya partial gastrectomy (eight years ago). Oral iron during all years after operation in intervalls.	14 9	135	*290*
302	A G	63	"	Previously chronic iron deficiency anemia from bleeding hemorrhoids. After hemorrhoidectomy and normalisation of the Hb value treated 3 months with oral iron.	14 4	116	*311*
303	N G	54	"	Chronic iron deficiency anemia with Coilonychia and achlorhydria. After 4 months of oral iron treatment	16.3	68	*391*
304	B A	Fm 43		Chronic iron deficiency anemia and achlorhydria After 4 months of oral iron	12.6	104	*311*

Fm = Menstruating women

References

1. Schmiedeberg, O. Ueber das Ferratin und seine diätetische und therapeutische Anwendung. Arch. exper. Pathol. u. Pharmakol. *33* 101 1894

2. Laufberger, M. V. Sur la cristallisation de la ferritine. Bull. Soc. chim. biol. *19* 1575, 1937

3. Kuhn, R., Sörensen, N. A., und Birkhofer, L. Über die Eisenproteide der Milz, der Beispiel des Ferritins, Ber. der dtsch. chem. Ges. *73* 823 1940

4. Granick, S. and Michaelis, L. Ferritin and apoferritin. Science 95, 439 1942.

5. Granick, S. Ferritin. I. Physical and chemical properties of horse spleen ferritin. J. Biol. Chem. *146* 451 1942.

6. Michaelis, L. Coryell, C. and Granick, S. Ferritin III. The magnetic properties of ferritin and some other colloidal ferric compounds. J. Biol. Chem. *148* 463, 1943.

7. Michaelis, L. In Physical Methods of Organic Chemistry Vol. II. Chapter on Magnetochemistry. Interscience Publishers, New York 1947

8. Granick, S. Ferritin its properties and significance for iron metabolism. Chem. Rev. *38* 379 1946.

9. Rothen, A. Ferritin and apoferritin in the ultracentrifuge. Studies on the relationships of ferritin and apoferritin; precision measurements of the rates of sedimentation of apoferritin. J. Biol. Chem. *152* 679 1944.

10. Mazur, A. Litt, I. and Shorr, E. Chemical properties of ferritin and their relation to its vasodepressor activity. J. Biol. Chem. *187* 473 1950

11. Harrison, P. Ferritin and Haemosiderin. in Iron Metabolism pp. 47. Ed. by F. Gross Springer. Berlin 1964

12. Farrant, J. L. An electron microscopic study of ferritin. Biochim. et biophys. acta *13* 569 1954

13. Richter, G. W. A study of hemosiderosis with the aid of electron microscopy with observations on the relationship between hemosiderin and ferritin. J. Exp. Med. *106* 203, 1957

14. Muir, A. R. The molecular structure of isolated and intracellular ferritin. Quart. J. Exp. Physiol. *45* 192, 1960.

15. Richter, G. W. On ferritin and its production by cells growing in Vitro. Lab. Invest. *12* 1026, 1963

16. Mazur, A. and Shorr, E. A quantitative immunochemical study of ferritin and its relation to the hepatic vasodepressor material. J. Biol. Chem. *182* 607 1950

17. Granick, S. Ferritin IV; occurrence and immunological properties of ferritin. J. Biol. Chem. *149* 157 1943.

18. Mazur, A. and Shorr, E. Hepatorenal factors in circulatory homeostasis. IX. The identification of the hepatic vasodepressor substance, VDM with ferritin. J. Biol. Chem. *176* 771 1948.

19. Wöhler, F. Bessis, H. J. Über die Natur des Hemosiderins. In Eisenstoffwechsel pp. 82—92. Georg Thieme, Stuttgart 1959

20. Wöhler, F. Über die Natur des Hämosiderins. Acta Haemat. *23* 342, 1960

21. Hofmann, T. Harrison, P. M. The structure of apoferritin; degradation into and molecular weight of subunits. J. Molec. Biol. *6* 256 1963.

22. McKay, R. H. and Fineberg, R. A.: Horse Spleen Hemosiderin. I. Isolation. Arch. Biochem. Biophys. *104* 487 1964.

23. Neumann, E.: Beiträge zur Kenntnis der pathologischen Pigmente. Virchow Arch. Path. Anat. *3* 25 1858.

24. Shoden, A., and Sturgeon, Ph. Hemosiderin. A physico-Chemical study. Acta Haemat. *23* 376 1960.

25. Behrens, M. and Asher, Th. Untersuchungen und Isolierung des Hämosiderins in der Pferdemilz. Ztschr. f. physiol. Chem. *220* 97 1933

26. Behrens, M. and Taubert, M. Über die Beziehung zwischen Hämosiderin und Ferritin. Ztschr. f. physiol. Chem. *289* 116, 1952.

27. Greenberg, D. M. Intermediary metabolism and biologic activities of ferritin. II. In Metabolism and function of iron. Report of the 19th Ross Pediatric Research Conference Ross Laboratories, Columbus, Ohio 1956, pp. 33—35

62. Gabrio, B. W., Shoden, A., Finch, C. A.: A quantitative fractionation of tissue ferritin and hemosiderin. J. Biol. Chem. 204 815, 1953.

63. Kaldor, I.: Measurements of the iron derived from water soluble and water insoluble non-haem compounds (ferritin and haemosiderin iron) in liver and spleen. Aust. J. Exp. Biol. Med. Sci. 36 173, 1958.

64. Mendel, H. E. und Sundermann, A.: Darstellung von Ferritin aus menschlicher Leber. Folia Haemat. 78 54, 1961.

65. Stenninger, Gunnar: Chemical and histochemical estimation of depot iron; Results in various blood disorders. Proc. 7th Congr. europ. Soc. Haemat. II/1, 1960, Karger Basel.

66. Kerr, L. M. H.: A Method for the Determination of non-haem iron in bone marrow. Biochem. J. 67 627, 1957.

67. Nordenson, N. G.: Studies on Bone Marrow from Sternal Puncture. Diss. Stockholm, 1935 Sweden.

68. [illegible], J. and Higgins, G. M.: A cytologic study of the marrow in flat bones of man. Folia Haemat. 61 334, 1939.

69. Barnicke, T., Gormsen, H. and Moller, B.: Comparative studies of bone marrow punctures of the sternum, the iliac crest and the spinous process. Acta Med. Scand. 177 155, 1956.

70. Davidson, J. N., Leslie, I., White, J. C.: The distribution of nucleic acids in bone marrow cells. Biochem. J. 41 xxvi, 1947.

71. Hoff-Jørgensen, E.: A microbiological assay of deoxyribonucleosides and deoxyribonucleic acid. Biochem. J. 50 400, 1951.

72. Lewin, S. and Roos, K.: Microbiological determination of DNA. The growth of Thermobacterium Acidophilum on chemically defined medium. Exp. Cell Res. Suppl. 4 269, 1957.

73. Lewin, S. and Roos, K.: Microbiological determination of deoxyribonucleic acid. Acta Biochim. Pol. 10 73, 1963.

74. Vendrely, R.: The deoxyribonucleic acid content of the nucleus. In The Nucleic Acids. Vol. II. Ed. by Chargaff and Davidson, Academic Press Inc. Publishers, New York, 1955.

75. Custer, R. P. and Ahlfeldt, F. E.: Studies on the structure and function of bone marrow. J. Lab. Clin. Med. 17 960, 1932.

76. Gross, H. S., Sandberg, M., Holly, M. O.: Changes in copper and iron retention in chronic diseases accompanied by secondary anemia. Am. J. Med. Sci. 204 201, 1942.

77. Sandberg, M., Gross, H., and Holly, O. M.: Changes in retention of copper and iron in liver and spleen in chronic diseases accompanied by secondary anemia. Arch. Path. 33 834, 1942.

78. Schaefer, E., Reichenberger, J.: Das Leber und Milzeisen bei Mann und Frau in verschiedenen Lebensaltern. Virchow Arch. Path. Anat. 315 309, 1948.

79. Morgan, E. H., and Walters, M. N. I.: Iron storage in human disease. Fractionation of hepatic and splenic iron into ferritin and haemosiderin with histochemical correlations. J. Clin. Path. 16 101, 1963.

80. B[illegible], G. and Zondek, S. G.: Iron, copper and manganese in human organs at various ages. Biochem. J. 33 1845, 1939.

81. McCance, R. A., and Widdowson, E. M.: The absorption and excretion of iron following oral and intravenous administration. J. Physiol. 94 148, 1938.

82. McCance, R. A., and Widdowson, E. M.: Iron excretion and metabolism in man. Nature 152 326, 1943.

83. Moore, C. V.: The importance of nutritional factors in the pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutr. 3 3, 1955.

84. Callender, T. S.: Physiology of Iron. Digestive absorption of iron. In Iron Metabolism. Edited by F. Gross. Springer, Berlin 1964.

85. Copp, D. H. and Greenberg, D. M.: A tracer study of iron metabolism with radioactive iron. I. Methods, absorption and excretion of iron. J. Biol. Chem. 164 377, 1946.

86. Bothwell, T. H., Pirzio-Biroli, G. and Finch, C. A.: Iron absorption. I. Factors influencing absorption. J. Lab. Clin. Med. 51 24, 1958.

87. Pirzio-Biroli, G. and Finch, C. A.: Iron absorption. III. The influence of iron stores on iron absorption in the normal subject. J. Lab. Clin. 55 216, 1960.

88. Laurell, C. B.: Studies on the iron transportation and metabolism of iron in the body. Acta Physiol. Scand. Vol. 14 Supp. 46, 1947.

89. Hallberg, L. and Sölvell, L.: Iron Absorption Studies. Acta Med. Scand. 168 Suppl. 358, 1960.

90. Charley, Ph. J., Stitt, C., Shore, E. and Saltman, P.: Studies in the regulation of intestinal iron absorption. J. Lab. Clin. Med. 61 397, 1963.

91. Pollack, S., Balcerzak, S. P., Crosby, W. H.: Transferrin and the absorption of iron. Blood 21 33, 1962.

92. Wheby, M. S. and [illegible], G. J.: Role of transferrin in iron absorption. J. Clin. Invest. 42 1007, 1963.

28. LUDEWIG, S Hemosiderin. Isolation from horse spleen and characterisation. Proc. Soc. Exp Biol. Med. *95* 514 1957

29 RICHTER, G W The *nature* of storage iron in idiopathic hemochromatosis and in hemosiderosis. J Exp Med. *112* 551 1960.

30 SCHWIETZER, C. H. Untersuchungen uber das Hemosiderin. Acta Haemat. *10* 174 1953

31 LUDEWIG, STEPHAN: Hemosiderin. Iron extraction studies. Proc. Soc. Exp Biol. Med. *100* 299 1959

32. MCKAY R. H. and FINEBERG R. A., Chemical studies on hemosiderin. Federation Proc. *17* *272*, 1958.

33 MCKAY R. H. and FINEBERG R. A., Horse *Spleen Hemosiderin. II Further Characterization*. Arch. Biochem. Biophys. *104* 496 1964

34 SHODEN A. and STURGEON PH., Formation of haemosiderin and its relation to ferritin. Nature *189* 846, 1961

35 GEDIGK, P., und STRAUSS, G Zur Histochemie des Hemosiderins. Virchow Arch. Path. Anat. *324* 373 1953

36 GEDIGK, P und STRAUSS, G Zur formalen Genese der Eisenpigmente. Virchow Arch. Path. Anat. *326* 172, 1954

37 *GEDIGK P Die funktionelle Bedeutung des* Eisenpigmentes. Ergebn. allg Path. *38* 1 1958

38. RICHTER, G W Electron microscopy of hemosiderin. Presence of ferritin and occurence of *crystalline lattices in hemosiderin* deposits. J Biophysic. Biochem. Cytol. *4* 55 1958.

39 RICHTER, G W The cellular transformation of injected colloidal iron complexes into ferritin and hemosiderin in experimental animals. *J Exp. Med. 109* 197 1959

40 BESSIS, M BRETON-GORIUS, J Différents aspects du fer dans l'organisme I Ferritine et micelles ferrugineuses. II Différentes formes de l'hémosidérine. J Biophys. Biochem Cytol. *6* 231 1959

41 BESSIS, M. C. and BRETON-GORIUS. Iron metabolism in the bone marrow as seen by electron microscopy: A critical review *Blood 19* 635 1962.

42. SHODEN A. and RICHTER, G W On Extraction and Staining of Ferritin. Folia Haemat. *4* 180 1960

43. BOGNIARD *R. P* and *WHIPPLE, G H* Iron content of blood free tissues and viscera. J Exp Med. *55* 653 1932.

44 AUSTONI, M. E., RABINOVITCH, A., and GREENBERG, D M. Iron content of tissues of normal, anemic and iron enriched rats freed from blood by viviperfusion. J Biol. Chem. *134* 17 1940.

45. SCHAEFER, K. H. Die Bestimmung des Eisens in biologischen Material, insbesondere des Nichthämoglobineisens in geringen Organ-*mengen durch Phenanthrolin als Indikator*. Biochem. Z. *304* 417 1940.

46 SCOTT E. M., The determination of iron in tissues. Arch. Biochem. *6* 27 1945

47 STARKENSTEIN, E., and WEDEN, H. Über das anorganische Eisen des Organismus, Arch. f. exper Path. u. Pharmakol. *134* 274 1928.

48. ANDERSSON, N. S E., Experimental and Clinical Investigations into the effect of parenterally administered iron. Acta Med. Scand. *138* Suppl. 241 1950

49 NYLANDER, G On placental transfer of iron experimental study in rat. Acta Physiol. Scand. Suppl. 107 *29* 1 1953.

50. HALLGREN, B., Haemoglobin formation and storage iron in protein deficiency Acta Soc. Med. Upsal. *19* 79 1953.

51 KALDOR, L. The measurement of non-hemoglobin tissue iron. Aust. J Exp. Biol. and Med. Sci. *32* 795 1954

52. THOMPSETT S. L., Copper and inorganic iron of human tissues. Biochem. J *29* 480 1935

53 B OCKMAN G and ZONDEK, S. G An Improved method for the determination of nonhemin iron. J Biol. Chem. *135* 23, 1940

54 ELVEHJEM C. A., HAR E. B and SHERMAN, W C. Availability of iron from different sources for hemoglobin formation, J Biol. Chem. *103* 61 1933.

55. SHERMAN, W C., ELVEHJEM, C. A., and HART E. B Factors influencing utilization of iron and copper of egg yolk for hemoglobin formation J Biol. Chem. *107* 289 1934

56. SHACKLETON, L., and MCCANCE, R. A.. Ionisable iron in foods. Biochem. J *30* 582, 1936.

57 BORGEN D R., and ELVEHJEM, C. A. Factors affecting determination of inorganic iron in animal tissues. J Biol. Chem. *119* 725, 1937

58. GALE, E., TORRANCE, J and BOTHWELL, T The quantitative estimation of total iron stores in human bone marrow J Clin. Invest. *42* 1076, 1963.

59 VENNDT H. Untersuchungen uber den Abbau des Hämoglobins bei Einwirkung verdunnter Säuren. Hoppe Seyler Z. Physiol. Chem. *263* 162, 1940.

60 SCOTT E. M. and MCCOY R. H. Iron in anemic rat tissues Arch. Biochem. *5* 349 1944

61 KEIDERLING, W und WOHLER, F Zur Physiologie und Pathologie des Speichereisens. Eine Methode zu quantitativen Bestimmung des Ferritins. Arch. exper Path. u. Pharmakol. *221* 418, 1954

62. Gabrio, B. W., Shoden, A., Finch, C. A. A quantitative fractionation of tissue ferritin and hemosiderin. J. Biol. Chem. 204 815, 1953.

63. Kaldor, L. Measurements of the iron derived from water soluble and water insoluble non-haem compounds (ferritin and haemosiderin iron) in liver and spleen. Aust. J. Exp. Biol. Med. Sci. 36 173, 1958.

64. Mendel, H. E. and Sundermann, A. Darstellung von Ferritin aus menschlicher Leber. Folia Haemat. 78 54, 1961.

65. Steinkamp, Gunnar. Chemical and histochemical estimation of depot iron. Results in various blood disorders. Proc. 7th Congr. europ. Soc. Haemat., II/1, 1960, Karger, Basel.

66. Kerr, L. M. H. A Method for the Determination of non-haem iron in bone marrow. Biochem. J. 67 627, 1957.

67. Nordenson, N. G. Studies on Bone Marrow from Sternal Puncture. Diss. Stockholm, 1935, Sweden.

68. Stasney, J. and Higgins, G. M. A cytologic study of the marrow in flat bones of man. Folia Haemat. 61 334, 1939.

69. Berlin, T., Gorstein, H. and Möller, B. Comparative studies of bone marrow punctures of the sternum, the iliac crest and the spinous process. Acta Med. Scand. 377 155, 1956.

70. Davidson, J. N., Leslie, I., White, J. C. The distribution of nucleic acids in bone marrow cells. Biochem. J. 41 xxvi, 1947.

71. Hoff-Jørgensen, E. A microbiological assay of deoxyribonucleosides and deoxyribonucleic acid. Biochem. J. 50 400, 1951.

72. Lewitus, S. and Roos, K. Microbiological determination of DNA. The growth of Thermobacterium Acidophilum on chemically defined medium. Exp. Cell Res. Suppl. 4 269, 1957.

73. Lewitus, S. and Roos, K. Microbiological determination of deoxyribonucleic acid. Acta Biochim. Pol. 10 73, 1963.

74. Vendrely, R. The deoxyribonucleic acid content of the nucleus. In The Nucleic Acids. Vol. II. Ed. by Chargaff and Davidson, Academic Press Inc., Publishers, New York, 1955.

75. Custer, R. P. and Ahlfeldt, F. Studies on the structure and function of bone marrow. J. Lab. Clin. Med. 17 960, 1932.

76. Gross, H., Sandberg, M., Holly, M. O. Changes in copper and iron retention in chronic diseases accompanied by secondary anemia. Am. J. Med. Sci. 204 201, 1942.

77. Sandberg, M., Gross, H., and Holly, O. M. Changes in retention of copper and iron in liver and spleen in chronic diseases accompanied by secondary anemia. Arch. Path. 33 834, 1942.

78. Schaefer, E., Rechenberger, J. Das Leber und Milzeisen bei Mann und Frau in verschiedenen Lebensaltern. Virchow Arch. Path. Anat. 315 309, 1948.

79. Morgan, E. H., and Walters, M. N. I. Iron storage in human disease. Fractionation of hepatic and splenic iron into ferritin and hemosiderin with histochemical correlations. J. Clin. Path. 16 101, 1963.

80. Butterman, G. and Zondek, S. G. Iron, copper, and manganese in human organs at various ages. Biochem. J. 33 1845, 1939.

81. McCance, R. A., and Widdowson, E. M. The absorption and excretion of iron following oral and intravenous administration. J. Physiol. 94 148, 1938.

82. McCance, R. A., and Widdowson, E. M. Iron excretion and metabolism in man. Nature 152 326, 1943.

83. Moore, C. V. The importance of nutritional factors in the pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutr. 3 3, 1955.

84. Callender, T. S. Physiology of Iron. Digestive absorption of iron. In Iron Metabolism. Edited by F. Gross. Springer, Berlin 1964.

85. Copp, D. H. and Greenberg, D. M. A tracer study of iron metabolism with radioactive iron. I. Methods, absorption and excretion of iron. J. Biol. Chem. 164 377, 1944.

86. Bothwell, T. H., Pirzio-Biroli, G. and Finch, C. A. Iron absorption. I. Factors influencing absorption. J. Lab. Clin. Med. 51 24, 1958.

87. Pirzio-Biroli, G., and Finch, C. A. Iron absorption III. The influence of iron stores on iron absorption in the normal subject. J. Lab. Clin. 55 216, 1960.

88. Laurell, C. B. Studies on the iron transportation and metabolism of iron in the body. Acta Physiol. Scand. Vol. 14 Supp. 46, 1947.

89. Hallberg, L. and Sölvell, L. Iron Absorption Studies. Acta Med. Scand. 168 Suppl., 358, 1960.

90. Charlet, Ph. J., Stitt, C., Shore, E. and Saltman, P. Studies in the regulation of intestinal iron absorption. J. Lab. Clin. Med. 61 397, 1963.

91. Pollack, S., Balcerzak, S. P., Crosby, W. H. Transferrin and the absorption of iron. Blood 21 33, 1962.

92. Wheby, M. S., and Jeffrey, G. J. Role of transferrin in iron absorption. J. Clin. Invest. 42 1007, 1963.

28 LUDEWIG S Hemosiderin. Isolation from horse spleen and characterization. Proc. Soc. Exp. Biol. Med. *95*:514 1957

29 RICHTER, G W The nature of storage iron in idiopathic hemochromatosis and in hemosiderosis. J Exp Med. *112*:551 1960

30 SCHWIETZER, C. H. Untersuchungen uber das *Hemosiderin. Acta Haemat. 10 174 1953*

31 LUDEWIG, STEPHANG Hemosiderin. Iron ex traction studies. Proc. Soc. Exp. Biol. Med. *100* 299 1959

3_ MCKAY R. H. and FINEBERG, R. A. Chemical *studies on hemosiderin.* Federation Proc. *17* 272, 1958.

33 MCKAY R. H and FINEBERG R. A. Horse Spleen Hemosiderin. II Further Characterization. Arch. Biochem. Biophys. *104*:496, 1964

34 SHODEN A. and STURGEON, PH. Formation of hemosiderin and its relation to ferritin. Nature *189* 846 1961

35 GEDIGK, P und STRAUSS, G Zur Histochemie des Hemosiderins. Virchow Arch. Path. Anat. *324*.373, 1953

36 GEDIGK, P und STRAUSS, G Zur formalen Genese der Eisenpigmente. Virchow Arch. Path. Anat. *326* 172, 1954

37 GEDIGK, P Die funktionelle Bedeutung des Eisenpigmentes. Ergebn. allg. Path. *38* 1 1958

38. RICHTER, G W Electron microscopy of hemosiderin: Presence of ferritin and occurence of crystalline lattices in hemosiderin deposits J Biophysic. Biochem. Cytol. *4*:55 1958.

39 RICHTER, G W The cellular transformation *of injected colloidal iron complexes into fer* ritin and hemosiderin in experimental animals. J Exp. Med. *109* 197 1959

40 BESSIS, M BRETON-GORIUS, J Différents aspects du fer dans l'organisme. I. Ferritine et micelles ferrugineuses. II. Différentes formes de l'hémosidérine. J Biophys. Biochem. Cytol. *6* 231 1959

41 BESSIS, M. C. and BRETON-GORIUS. Iron metabolism in the bone marrow as seen by elec tron microscopy A critical review Blood *19*:635 1962

42. SHODEN A. and RICHTER, G W On Extrac tion and Staining of Ferritin. Folia Hae mat. *4* 180 1960.

43 BOGNIARD, R. P and WHIPPLE, G H Iron content of blood free tissues and viscera J Exp Med. *55* 653, 1932.

44 AUSTONI, M. E., RABINOVITCH, A and GREENBERG, D M. Iron content of tissues of nor mal, anemic and iron enriched rats freed from blood by hyperfusion. J Biol. Chem. *134* 17 1940.

45 SCHAEFER, K. H. Die Bestimmung des Eisens in biologischen Material, insbesondere des Nichthämoglobineisens in geringen Organmengen durch Phenanthrolin als Indikator. Biochem. Z. *304*:417 1940

46. SCOTT E. M The determination of iron in tissues. Arch. Biochem. *6* 27 1945

47 STARKENSTEIN E., and WEDEN, H. Über das anorganische Eisen des Organismus, Arch. f exper Path. u. Pharmakol. *134*.274 1928.

48. ANDERSSON, N. S. E. Experimental and Clinical In estigations into the effect of parenterally administered iron. Acta Med. Scand. *138* Suppl. 241 1950.

49 NYLANDER, G On placental transfer of iron experimental study in rat. Acta Physiol. Scand. Suppl. 107 *29* 1 1953

50 HALLGREN, B. Haemoglobin formation and storage iron in protein deficiency Acta Soc. Med. Upsal. *19* 79 1953

51 KALDOR, I The measurement of non-hemoglobin tissue iron. Aust. J Exp. Biol. and *Med. Sci. 32 793 1954*

5_ T OMPKETT S. L. Copper and "inorganic" iron of human tissues. Biochem. J *29*:480, 1935.

53. BRUCKMAN, G., and ZONDEK, S. G An Im*proved method for the determination of non*hemin iron. J Biol. Chem. *135*.23 1940.

54 ELVEHJEM C. A., HART E. B and SHERMAN, W C. Availability of iron from different sources for hemoglobin formation, J Biol. Chem. *103*:61 1933.

55 SHERMA , W C., ELVEHJEM, C. A., and HA T E. B Factors influencing utilization of iron and copper of egg yolk *for hemoglobin for* mation. J Biol. Chem. *107* 289 1934

56. S ACKLETON L., and MCCANCE, R. A. Ionizable iron in foods. Biochem. J *30*:582, 1936.

57 BORGEN D R., and ELVEHJEM, C. A. Factors *affecting* determination of inorganic iron in animal tissues. J Biol. Chem. *119* 725 1937

58. GALE, E., TORRANCE, J and BOTHWELL, T The quantitativ estimation of total *iron* stores in human bone marrow J Clin. Invest. *42* 1076, 1963

59 VENNDT H. Untersuchungen bei den Abba des Hämoglobins bei Einwirkung ver dunnter Säuren. Hoppe Seyler Z. Physiol Chem. *263* 162, 1940.

60 SCOTT E. M and MCCOY R. H. Iron in ane mic rat tissues Arch. *Biochem. 5* 349 1944

61 KEIDERLING, W und WÖHLER, F Zur Physiologie und Pathologie des Speichereisens. Eine Methode zu quantitativen *Bestimmung* des Ferritins. Arch. exper Path. u. Pharmakol. *21*:418, 19_4

124 Weinfeld, A., Hansen, H. A., Further studies on the interrelationship between hemosiderin and sideroblasts in bone marrow smears. Acta Med. Scand. 171:23, 1962.

125. Haskins, D., Stevens, A. R., Jr., Finch, S. C., and Finch, C. A., Iron metabolism. Iron stores as measured by phlebotomy. J. Clin. Invest. 31:543, 1952.

126. Mechanik, N.: Untersuchungen über das Gewicht des Knochenmarkes des Menschen. Z. Anat. Entwicklungsgesch. 79:52, 1925.

127 Weinfeld, A.: Non-haemin Iron, serum iron and TIBC in relation to time after the Poly partial gastrectomy in non-anemic, male subjects. Report Swed. Soc. Intern. Med. Stockholm, 1963. To be published.

128 Cartwright, G. E., Lauritsen, M. A., Humphreys, S., Jones, P. J., Merrill, L. M., Wintrobe, M. M., Anemia of infection. I Hypoferremia, hypercupremia, and alterations in porphyrin metabolism in patients. J. Clin. Invest. 25:65, 1946.

129 Cartwright, G. E. and Wintrobe, M. M. The Anemia of Infection: A Review. Advances Intern. Med. Vol. V. Year Book Publishers, 1952.

130. Vanotti, A., Sur les causes des anémies infectieuses. Rev. méd. Suisse Rom. 69:712, 1949.

131 Heilmeyer, L., Keiderling, W., and Stüwe, G., Kupfer und Eisen als körpereigene Wirkstoffe und ihre Bedeutung beim Krankheitsgeschehen. Jena, Gustav Fischer, 1941.

132 Hevesy, G. and Lockner, D., Iron metabolism in health and in the neoplastic state. Arkiv För Kemi 19:303, 1962.

133. Miller, A., Chodos, R. B., Emerson, C. P., and Ross, J. F. Studies of the anemia and iron metabolism in cancer. J. Clin. Invest. 35:1248, 1956.

134 Bush, J. A., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M. The Anemia of Infection. XX. The kinetics of iron metabolism in the anemia associated with chronic infection. J. Clin. Invest. 35:89, 1956.

135 Schäfer, K. H. Über den Einfluss von Infektionen und ähnlichen Vorgängen auf den Eisenstoffwechsel. Z. Ges. Exp. Med. 110:678, 1942.

136 Nilsson, F. Anemia problems in Rheumatoid arthritis. Acta Med. Scand. Vol. 130. Suppl. 210, 1948.

137 Heilmeyer, L., Keiderling, W. and Wöhler, F. Der Eisenstoffwechsel beim Infekt und die Entgiftungsfunktion des Speichereisens. Deutsche Med. Wschr. 83:1965, 1958.

138. Keiderling, W., Schmidt, H. A. E.: Die Infektiöse und neoplastische Eisenstoffwechselstörung. Eisenstoffwechsel, Georg Thieme, Stuttgart, 1959.

139 Greenberg, C. J., Cartwright, G. E. and Wintrobe, M. M.: The anemia of infection. X. The effect of the infection on the absorption and storage of iron by the rat. J. Biol. Chem. 184:563, 1950.

140. Schäfer, K. H., Breuer, A., Experimentelle Untersuchungen zur Frage der Eisenresorption im Infekt. Klin. Wschr. 34:64, 1956.

141 Keiderling, W., Diechter, W.: Die Eisenresorption beim Infekt. Deutsch. Med. Wschr. 15:719, 1964.

142. Dubach, R., Callender, S. T. E., and Moore, C. V. Studies in Iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anemias of varied etiology. Blood 3:526, 1948.

143. Jeffrey, M. R., Freundlich, H. F., Jackson, E. B., and Watson, D. The absorption and utilization of radioiron in rheumatoid arthritis. Clin. Sci. 14:395, 1955.

144 Heilmeyer, L. and Wöhler, F. Über die Entgiftungsfunktion des Speichereisens. Klin. Wschr. 39:563, 1961.

145. Pechet, G. S., and MacDonald, R. A. Idiopathic hemosiderosis: Relation to idiopathic hemochromatosis. New Engl. J. Med. 267:6, 1962.

146. MacDonald, R. A. and Pechet, G. S.: Liver and tissue iron. Arch. Path. 77:348, 1964.

147 Reichenmiller, J. and Schaible, E., Leber und Milzeisen bei verschiedenen Infektionskrankheiten. Virchow Arch. Path. Anat. 315:326, 1948.

148. Aron, E., Gautier, C., Paoletti, C., Jobard, P., Gosse, Ch., Berthelot et Mlle J. Buisson: La responsabilité Du Fer Viscéral dans la Cytosidérose Hépatique. Rev. Méd. Tours. 177, 1961.

149 Shoden, A., and Sturgeon, Ph. Iron Storage. I. The influence of time on the redistribution of excess storage iron. Amer. J. Path. 34:1139, 1958.

150. Shoden, A. and Sturgeon, Ph. Iron Storage. II. The influence of the type of compound administered on the distribution of iron between ferritin and hemosiderin. Acta Haemat. 22:140, 1959.

151 Shoden, A., and Sturgeon, Ph. Iron Storage III. The influence of rates of administration of iron on its distribution between ferritin and hemosiderin. Acta Haemat. 27:33, 1962.

93 VAHLQUIST B Normalvärden for hämoglobin, röda blodkroppar och "index" ut tryckta i absoluta tal för olika åldrar Svenska Läkartidning *45* 2471, 1948.

94 *COLEMAN D H, STEVENS A R*, and FINCH C A. The treatment of iron deficiency anemia. Blood *10* 567 1955

95 STEVENS, A. R., JR., The mechanism and treatment of iron deficiency anemia. Arch. Intern Med. *95* 550 1956

96 CONRAD, M. E., and CROSBY W H., The natural history of iron deficiency induced by phlebotomy Blood *20* 173 1962.

97 *DRABKIN, D L*. Spectroscopy photometry and spectrophotometry In. Medical Physics. Edited by Glaser O The Yes Book Publishers, Inc. Chicago, 1954.

98. LAURELL, C. B Central Laboratory Malmo, Allmänna sjukhus: Personal communication.

99 PETERS T., GIOVANIELLO, T J APT L., ROSS, J F A new method for the determination of serum iron-binding capacity I J Lab Clin. Med. *48* 274 1956.

100 SNEDECOR, G W Statistical Methods. Iowa State University Press Iowa 1956.

101 EDLUND Y and ZETTERGREN L. Histopathology of the gallbladder in gallstone disease related to clinical data with a proposal for uniform surgical and clinical terminology *Acta Chir Scand. 116* 450 1958/59

102. MEIER, W BENEKE, G A LLER G Die Veränderungen on Ferritin in Relation zum Hämosiderin und Gesamteisen der Leber beim Mann in den verschiedenen Alters stufen Z. Ges. Inn. Med. *14* 1065 1959

103 BOTHWELL, T H and FINCH C A. Iron *Metabolism. Little Brown, 1962*.

104 ROTH, O JASINS I B. and v BUDDER, H. Das Gewebeeisen beim Menschen bei normalen und pathologischen Zuständen. Hel Med. Acta. *18* 159 1951

105 LINTZEL, W RECHENBERGER, J and SCHA AER, E. Über den Eisenstoffwechsel des Neugeborenen und des Säuglings Z. Ges. Exp Med. *113* 591 1944

106. SMITH, N J ROSELLO S SA M B. YETS, K. Iron storage in the first years of lif Pediatrics *16* 166, 1955

107 BEINKE, A R. J The *Liver relation to* the body as a whole Li er Function. Edited by Brauer R. W American Institute of Biological Sciences Washington D C., 1958

108. BÜRGER, M Altern und Krankheit XX Ln Thieme, Lpz. 1957

109 FINCH, S. HASKINS, D and FINCH C A Iron metabolism, hematopoesis following phlebotomy Iron as limiting factor J Clin. Invest. *29* 1078 1950.

110. COOKE, W T., FRAZER, A. A C., PEENEY A L. P., SAMMONS, H. G and THOMPSON, M. D. Anomalies of intestinal absorption of fat. II The haematology of idiopathic steatorrhoea. Quart. J Med. *17* 9 1948.

111 BADENOCH, J CALLENDER, S T Iron metabolism in steatorrhea. The use of radioactive iron in studies of absorption and utilization Blood *9* 123 1954.

112. LUBLIN H., On the late symptoms after gastroenterostomy and resection of the stomach (*Billroth II*) for gastric and duodenal ulcer Acta Med. Scand. Suppl. 41 1931

113 LYNGAR, E., Blood changes after partial gastrectomy for ulcer Acta Med. Scand. Suppl. 247 1950.

114 WALLENSTEN S., Results of the surgical treatment of peptic ulcer by partial gastrectomy according to Billroth I and II methods. Acta Chir Scand. Suppl. 191 1954

115 BAIRD I. M BLACKBURN, E. K., and WILSON G M., The pathogenesis of anemia after partial gastrectomy I Development of anemia in relation to time after operation, blood loss and diet. Quart. J Med. *24 21* 1959

116. SHODEN A., GABRIO, B. W and FINCH, C. A., The relationship between ferritin and hemosiderin in rabbits and man. J Biol. Chem. *204* 823 1953.

117 BEUTLER, E. FAIRBANKS, V F FAHEY J L. Clinical Disorders of Iron Metabolism. Grune & Stratton New York 1963

118. STEVENS, A. R., JR., COLEMAN D H., and FINCH C. A.. *Iron metabolism. Clinical* evaluation of iron stores. Ann. Intern. Med. *38* 199 1953.

119 BEUTLER, E., ROBSON M J and BUTTENWIESER, E.. A Comparison of the plasma iron, iron binding capacity sternal marrow iron and other methods in the clinical evaluation of iron stores. Ann. Intern. *Med. 48* 60, 1958

120. HANSEN, H. A. and WEINFELD A.. Hemosiderin estimations and sideroblast counts in the differential diagnosis of iron deficiency and other anemias. Acta Med Scand. *165* 333 1959

121 DOUGLAS, A. S and DACIE, J V The incidence and significance of iron-containing granules in human erythrocytes and their *precursors. J Clin. Path. 6* 307 1953.

122. DAVIDSSON, W L., and JENNISON R. F The relationship between iron storage and anemia. J Clin. Path. *5* 281 1952.

123 HUTCHISON, H. E., The significance of stainable iron in *sternal marrow sections. Blood 8* 236 1953

180. Mitchell, J. Haldin, M. R., Jones, F. Bayan, B. A., Sturm ×, J. A. and Meta man, E. E. Lowering of transferrin during iron absorption in iron deficiency. J. Lab. Clin. Med. 56:555 1960.

181. J ndo, J. Ivor ×, J. K., Samsova, R. L. and Allen, D. W. Transfer of iron from serum iron-binding protein to human reticulocytes. J. Clin. Invest. 38 161, 1959

182. Rundles, R. W. and Falls, H. F. Hereditary (? sex-linked) anemia. Am. J. M. Sci. 211 641 1946.

183. Wallerstein, R. O. and Aggeler, P. M. Anemia: Differentiating between thalassemia minor and iron deficiency. California Med. 84:176, 1956.

184. Heilmeyer, L., Plötner, K. Das Serumeisen und die Eisenmangelkrankheit. Jena. Gust Fischer, 1937

185. Waldenström, J. Iron and epithelium. Some clinical observations. Acta Med. Scand Suppl. 90:380, 1938.

186. Waldenström. J. Incidence of "iron deficiency" (sideropenia) in some rural and urban populations. Acta Med. Scand. Suppl. 170 252, 1946.

187 Rath, C. E. and Finch, C. A.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXVIII. Serum iron transport. Measurement of iron-binding capacity of serum in man. J. Clin. Invest. 28 79 1949

188. Cartwright, C. E., and Wintrobe, M. M. Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXIX. The anemia of infection. Studies on the iron-binding capacity of serum. J. Clin. Invest. 28:86, 1949

189 Rath, C. E. and Finch C. A. Sternal marrow hemosiderin: A method for the determination of available iron stores in man. J. Lab. Clin. Med. 33:81 1948.

190. Kaplan, E., Zuelzer, W. W. and Mouriquand, C.: Sideroblasts. A study of stainable non-hemoglobin iron in marrow normoblasts. Blood 9:203 1954

191 Moore, W. L. and Read, H. C. Stainable iron in marrow cells and erythrocytes in anemia. Canad. Serv. M. J. 10:244 1954

192. Lundin, P. Persson, E., Weinfeld, A. Comparison of hemosiderin estimation in bone marrow sections and bone marrow smears. Acta Med. Scand. 175:383, 1964

193. Pollycove, M. and Mortimer, R. The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects. J. Clin. Inv. 40 753 1961

194. Pollycove, M. Iron kinetics. In Iron Metabolism. Edited by Gross. Springer Berlin, 1964.

195. Pollycove, M.: Personal communication.

196. Bainton, D. F. Finch, C. A. The diagnosis of iron deficiency anemia. American J. of Med. 37:62, 1964.

197 Morgan, E. H. and Laurell, C. B. Studies on the exchange of iron between transferrin and reticulocytes. Brit. J. Haemat. 9:471 1963

198. Katz, J. H. and Jandl, J. H. The role of transferrin in the transport of iron into the developing red cell. In: Iron Metabolism. Ed. by F. Gross, Springer Berlin, 1964.

199 Mouriquand, C., Lejeune, E., Gramain, D et Matthieu, J. Le Fer Medullaire Dans Les Maladies Rheumatismales. Nouvelle Revue Française d'Hématologie 2:55, 1962.

152. SHODEN A., and STURGEON, PH. On the Formation of Haemosiderin and its relation to Ferritin. II. A radioisotopic Study. Brit. J Haemat. 9·513 1963

153 KEIDERLING W WOHLER, F und ALTMEYER, H. Zur Physiologie und Pathologie des Speichereisens. Arch. exp. Path. Pharmakol. 223 375 1954

154 HEILMEYER, L., WÖHLER, F und KEIDERLING, W. Neue Methode zur quantitativen Bestimmung von Ferritin in den Organen und ihre vorläufigen Ergebnisse. Acta Haemat. 12 154 1954

155 HEILMEYER, L. Ferritinstudien. Deutsch. Med. Wschr 80 2, 1577 1955

156 MORGAN, E. H. Iron storage and transport after administration of viable erythrocytes to the albino rat. Aust. J Exp. Biol. Med. Sci. 39 361 1961

157 HEILMEYER, L. Ferritin. In Iron in Clinical Medicine. Edited by Wallerstein and Mettier University of California Press, Berkeley

158. WOHLER, F., OTTE, W und FULGRAFF M. Zu Pathologie des Speichereisens. I. Mitteilung: Ferritin und Hemosiderin menschlicher Organe bei Leber Nieren und Kreislaufserkrankungen. Acta Haemat. 25.337 1961

159 WOHLER, F und OTTE, W II. Mitteilung: Ferritin und Hemosideringehalt menschlicher Organe bei entzündlichen Erkrankungen, Geschwulsten und Krankheiten des blutbildenden Systems. Acta Haemat. 26·81 1961

160 KENT G VOLINI F I., ORFEI, E., MINICK, O T DE LA HUERGA, J Effect of Hepatic Injuries upon Iron Storage in the Liver Lab. Invest. 12 1094 1963

KENT G MINICK, O T VOLINI, F I ORFEI, E., DE LA HUERGA, J Iron Storage in N 2 Fluorenylacetamide-Induced Hepatic Injury Lab. Invest. 12 1102, 1963

161 MORGAN E. H. Experimental Hyposiderosis. in Iron Metabolism. Edited by F Gross. Springer, Berlin 1964

162 POLICARD A., and BESSIS, M. Sur un mode d incorporation des macromolécules par la cellule, visible au microscope électronique la rophéocytose C. R. Acad. Sci 246 3194 1958

163 REISSMANN K. R and DIETRICH, M R. On the presence of ferritin in the peripheral blood of patients with hepatocellular disease. J Clin Invest 35·533 1956

164 HAGBERG, B. The iron-binding capacity of serum in infants and children. Acta Paediat. 42·589 1953

165. LAURELL, C B. Plasma iron and the transport of iron in the organism. Pharmacol. Rev 4 371 1952

166 FAY J CARTWRIGHT G E., and WINTROBE, M. M. Studies on free erythrocyte protoporphyrin, serum iron, serum iron-binding capacity and plasma copper during normal pregnancy J Clin. Invest. 28·487 1949

167 VENTURA, S. and KLOPPER, A. Iron metabolism in pregnancy J Obstet. Gynaec. Brit. Emp. 58 173 1951

168. BRENDSTRUP P Serum iron, serum copper and total iron-binding capacity of serum and serum copper in acute hepatitis. Acta Med. Scand. 146 107—113 1953

169 LAURELL, C. B. Iron Transportation. In Iron in Clinical Medicine. Edited by Wallerstein and Mettier. University of California Press, Berkeley 1956

170 CARTWRIGHT G E., and WINTROBE, M. M. Chemical clinical and immunological studies on the products of human plasma fractionation. XXXIX The anemia of infection. Studies on the iron-binding capacity of serum. J Clin. Invest. 28·86 1949

171 BRENDSTRUP P Serum copper serum iron and total iron-binding capacity of serum in patients with chronic rheumatoid arthritis. Acta Med. Scand. 146 384 1953.

172. LAHEY M. E., BEHAR, M VITERI, F and SCRIMSHAW N S Values for copper, iron and iron-binding capacity in the serum in kwashiorkor Pediatrics 22 72, 1958.

173 RIFKIND, D KRAVETZ, H. M., KNIGHT V and SCHADE, A. L. Urinary excretion of iron-binding protein in the nephrotic syndrome. New Engl. J Med. 265 115 1961

174 FINCH, S. C. and FINCH C. A. Idiopathic hemochromatosis, an iron storage disease Medicine. 34 381 1955

175 HAGBERG, B., WALLENIUS, G and WRANNE, L Latent iron deficiency after repeated removal of blood in blood donors. Scand. J Clin Lab. Invest. 10·63, 1958.

176 WEINFELD A. Iron stores in blood donors after intravenous and after prolonged oral iron administration determined by vigorous phlebotomy Rept. Swed. Soc. Int. Med Goteborg 1963. To be published.

177 RECHENBERGER, J Über die Eisenbindungskapazität des Blutserums, das Verhalten der Eisenbindungskapazität und des Serumeisens bei der unbehandelten und regenerierenden perniziösen Anämie. Deutsch. Z Verdau. Stoffwechselkr 16 223 1956.

178 MOORE, C. V., Iron metabolism and nutrition. Harvey Lect. 55·67 1959—60.

179 AWAI M and BROWN, E. B. Studies on the metabolism of J 131-labelled human transferrin. J Lab. Clin. Med. 61.363, 1963.

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SUPPLEMENTUM 426

A CLINICAL AND HEMODYNAMIC ANALYSIS OF FACTORS LIMITING THE CARDIAC PERFORMANCE IN PATIENTS WITH CORONARY HEART DISEASE

By

ROBERT O. MALMBORG

Accompanies Vol. 177

STOCKHOLM 1965

From the Department of Clinical Physiology and the Department of Medicine, Karolinska Institutet at Serafimerlasarettet, and the Department of Roentgenology Karolinska Institutet at Thorax kliniken, Karolinska Sjukhuset, Stockholm, Sweden.

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Chapter V

in collaboration with Björn Nordenström and Gunnar Törnell

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I

INTRODUCTION

Patients with coronary heart disease (i.e., atherosclerosis in the coronary arteries) with symptoms and signs of coronary insufficiency usually are restricted in their ability to perform physical work. In many cases this is due to congestive heart failure (i.e. marked dyspnea on effort, orthopnea, peripheral edema, roentgenological cardiac enlargement and pulmonary congestion) (Solem et al. 1963). Among patients with coronary heart disease without such symptoms and signs it can be postulated that the restriction can be due to other factors, altering the cardiac performance during exercise. These factors could be chest pain, or hypokinetic circulatory conditions (defined as a low cardiac output in relation to the oxygen consumption) with or without acute ventricular failure (registered as an abnormal relation between the intracardiac pressures and the blood flow) (Harvey 1962).

Purpose of the study

This study was undertaken to analyze the relative occurrence and significance of these postulated factors through correlation of the hemodynamic, clinical and laboratory findings in an appropriate group of patients with coronary heart disease. A group of patients with definite symptoms and signs of obstructive coronary artery disease, without clinical or roentgenological signs of congestive heart failure was selected. The following investigative procedures were used.

1) The individual case histories were analyzed, and pertinent clinical data were recorded.

2) Laboratory data regarding the total amount of hemoglobin, heart volume, ECG at rest, during and after exercise and exercise tolerance as well as coronary angiography were recorded.

3) Hemodynamic studies with measurements of flow and pressures in the systemic and pulmonary circulation, at rest and during exercise, before and after digitalization, were undertaken.

4) A control material consisting of subjects without significant cardiovascular disorders was selected, analyzed and studied in essentially the same manner as the patient group.

5) The results of the clinical and hemodynamic investigations were statistically analyzed both separately and conjointly.

1

INTRODUCTION

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II

PREVIOUS WORKS

Studies of the central hemodynamics on patients with coronary heart disease have previously been performed almost exclusively on patients with congestive heart failure and/or cardiac enlargement (Harvey et al. 1949 1951) (Bayliss et al. 1950) (Johnson et al 1959) (Ferrer et al 1960) (Selzer et al. 1960)

In a report on the hemodynamic consequences of angina pectoris Muller and Rorvik (1958) *however, presented results from* cardiac catheterization of patients with coronary heart disease both with and without cardiac enlargement. These studies included mild exercise periods aimed at precipitating *angina pectoris. In eleven male patients 45—*72 years of age with normal sized hearts (360—500 ml/m BSA) such exercise precipitated angina pectoris and pressure elevations in the lesser circulation suggesting simultaneous left ventricular failure The effect of nitroglycerin upon the pressure flow conditions in the central circulation during exercise was studied in four of these cases. After nitroglycerin the pressure in the pulmonary artery during a similar exercise load was lower than observed during the initial exercise and the patients did not develop angina pectoris.

Selzer and Malmborg (1962) while investigating the hemodynamic effects of Digoxin® *in latent cardiac failure, studied* eight patients with coronary heart disease who had recovered from an episode of congestive heart failure. In spite of normal sized hearts and no other symptoms or signs of heart failure, these patients had abnormally low cardiac and *stroke indices* and *high pulmonary* artery and pulmonary capillary venous pressures during mild exercise After acute intravenous digitalization of six of these patients the cardiac indices and stroke indices were *higher both at rest and during exercise* and the pressures in the pulmonary artery and pulmonary capillaries significantly lower during exercise.

Gorlin and associates while simultaneously *studying the coronary circulation at rest and* during mild exercise measured cardiac output, stroke volume, pulmonary artery and pulmonary capillary venous pressure in nineteen patients with coronary heart disease without congestive heart failure (Messer et al. 1963) The results were compared with similar studies in sixteen subjects with minimal heart disease (control subjects) (Gorlin et al. 1964) The authors found *significantly lower cardiac and stroke indices* both at rest and during exercise for the patients with coronary heart disease than for the control subjects. Despite the occurrence of angina pectoris during exercise none of *their patients developed* any significant pressure elevations in the pulmonary artery

MATERIAL

Thirty-eight male patients, 40 to 58 years of age with signs and symptoms of coronary insufficiency of varying degree (patients) and eleven male individuals, 39 to 56 years of age in whom no significant cardiovascular abnormality could be detected (controls) were examined.

A. Patients

The patients were hospitalized at Serafimerlasarettet during 1961—1964 with the principle diagnosis of coronary heart disease (Code no 420 in the Swedish Medical Board classification system)

Selection

The following criteria for the diagnosis of coronary heart disease were used.

1 Previous hospitalization for acute myocardial infarction with ECG changes indicative of myocardial damage and a blood transaminase pattern, suggestive of myocardial cell destruction (Agress, Wroblewski et al 1955) (Bjorck, Hansson 1956) (Broch J 1963)

2 History of angina pectoris according to the WHO criteria established by Rose (1962) in association with electrocardiographic abnormalities considered to be typical for coronary insufficiency These were ST J segment depressions of more than 1 mm with horizontal or downward sloping ST segment in the absence of signs of LVH present at rest, appearing or augmented during exercise, and present or accentuated 3 min after exercise (Lepeschkin 1960) (Mattingly 1962)

In order to allow a meaningful comparison between the clinical and the hemodynamic findings in patients with predominant coronary heart disease, individuals with the following abnormalities were not included Pulmonary dysfunction, valvular heart disease, a history of previous or present clinical evidence of congestive heart failure at rest, a cardiac volume of more than 530 ml/m² BSA, clinically diagnosed and treated arterial hypertension with grade III—IV fundi according to Keith, Wagener Barker (1939) atrial fibrillation, electrocardiographic signs of bundle branch block or left ventricular hypertrophy (Sokolow and Lyon 1949 Selzer et al. 1958) None of the studied patients received or had received digitalis preparations in the six weeks prior to the present study

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Clinical and laboratory data.

Individual heights, body weights and body surface areas according to the Du Bois formula, relativ heart olumes (ml/m² BSA) absolute heart olumes in the recumbent

position, total amounts of hemoglobin and resting blood pressures were measured and are listed in table 1 (see Appendix) together with the pertinent clinical history in each case. The resting brachial artery blood pressures were measured by the cuff method during complete bed rest in the morning

Case histories

The past and present disease history was analyzed by the investigator using all available hospital records and a personal interview

During this analysis special attention was paid to the following factors

1 *Degree of past or present physical activity* The individual degree of physical activity during daily life prior to the onset of symptoms was evaluated during the interview The subjects were divided in three different groups. Subjects with a previous low degree of physical activity were included in Group I Subjects with moderately strenuous physical activities were put in Group II Group III consisted of subjects with strenuous activities. In the present material fifteen of the patients belonged to group I, nine to group II and fourteen to group III

2. *Functional classification according to the New York Heart Association* The functional capacity limited by angina pectoris at the time of study was established during the personal interview and the individuals were grouped in the four (I—IV) classes for functional cardiac capacity revised in 1953 by the New York Heart Association

The definitions for these groups and the number in each from the material were as follows

Class I Patients with cardiac disease but without resulting limitation of physical activity Ordinary physical activity did not cause undue fatigue, palpitation, dyspnea or anginal pain. (Five cases)

Class II Patients with cardiac disease resulting in slight limitation of physical activity Ordinary physical activity resulted in fatigue, palpitation, dyspnea or anginal pain. (Eighteen cases)

Class III. Patients with cardiac disease resulting in marked limitation of physical activity Less than ordinary activity caused fatigue, palpitation, dyspnea or anginal pain (Fourteen cases)

Class IV Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome could be present even at rest. If any physical activity was undertaken discomfort was increased (One case)

3 *Time interval since most recent episode of myocardial infarction* The time interval between the last episode of acute infarction and the study was recorded (see Table 1) None of the patients had experienced an infarction less than six months previously The actual interval varied between six months and nine years.

4 *Number of previous myocardial infarctions* In evaluation of the records of previous hospitalizations special reference was made to the number of instances with definite signs of myocardial infarction. Only episodes with biochemical and/or electrocardiographic changes, diagnostic of acute myocardial necrosis were accepted as proven. Nineteen of the studied patients had been hospitalized for one or more proven acute myocardial infarctions. The remaining nineteen had angina pectoris only

The number of episodes of myocardial infarction in each individual varied from one

to four. One patient had been hospitalized four times for acute infarction, two patients three times, one patient twice and fifteen patients once only.

5. *Duration of symptoms*. The approximate duration of symptoms was established by recording of the earliest typical episode of angina pectoris or the date of the first infarction. In the present material the duration of symptoms varied between 4 months and more than 10 years.

6. *Type of symptoms*. Angina pectoris at rest, spontaneous or induced by physical activity and/or mental stress was the most common symptom of coronary insufficiency in this study. From the case histories angina pectoris induced by anxiety was found to be present in fifteen cases, and was found *during exercise in all cases examined*. Thirteen patients also complained of exertional dyspnea.

Individual anthropometric, occupational and clinical data are listed in table 1. (see Appendix)

B. Control subjects

Eleven men were included in the control group, and were studied with the same technique as the patients.

Seven of the control subjects were clinically healthy volunteers between 50 and 56 years of age with no abnormal findings at a routine physical examination and with ESR, routine blood and urine analysis, pulmonary function, roentgenologically determined heart size and shape and ECG:s at rest, during and after exercise all within normal limits. Also included in the control group were four men who had been admitted to the hospital for cardiological work up but in whom no abnormal findings were detected. One was admitted because of cardiac neurosis, two because of functional murmurs and one because of questionable bouts of paroxysmal tachycardia. The individual occupational and clinical data are listed in table 2. (see Appendix)

C. Comments

The selection of the patients included in the present study was based upon one basic principle, essential to the whole study, namely to obtain a material of patients with unequivocal symptoms and signs of coronary atherosclerosis without any other significant cardiovascular or pulmonary abnormalities that could interfere with the interpretation of the results and the clinical correlations. The criteria used for the selection have therefore been strict and the number of patients included in the study is relatively small. In men the earliest signs of coronary heart disease usually occur in the fifth to sixth decade of life. Strandell (1964, A) furthermore has recently demonstrated, that the normal aging process produces changes in the circulatory system that could obscure the analyses of hemodynamic data in patients with heart disease above 60 years of age. On the basis of these findings the present material was primarily selected from patients

40—60 years of age fulfilling all criteria outlined above.

In spite of the strict criteria the present group of subjects was heterogenous in several respects The number of previous clinically recognized infarctions varied between one and four among the patients with a diagnosis of myocardial infarction. Among the nineteen patients with a diagnosis of only angina pectoris, six were found to have initial QRS vector deflections suggestive of previous clinically silent myocardial infarctions Therefore the division of the patients into these two groups was rather arbitrary There was also considerable variation in the duration of symptoms ranging from about 6 months in some cases to more than 10 years in three cases

The association between coronary heart disease and hypertensive cardiovascular disease is well established (Biörck et al. 1958) (Wahlberg 1963) It is also often noted that a permanent arterial hypertension disappears after a myocardial infarction. The present material was probably heterogenous as to the presence of previous hypertension. As it was impossible to evaluate this factor it was instead minimized by exclusion of all patients with electrocardiographic signs of left ventricular hypertrophy and fundus hypertonicus III—IV

It is important that a control group used for comparison with patients does not include only subjects with large circulatory capacities and consequently supernormal hemodynamic variables The subject's physical fitness is therefore of importance for the selection of a suitable control group The present control group consisted of men of varying body size with different degrees of daily physical activity and with physical working capacities varying between 600 and 1500 kpm/min. The volunteers were from fire brigades, but only two (no 41 and 45) of them were still on active duty as firemen and thus perhaps more physically fit than the rest of the group The least physically fit in the control group was a man (no 39) referred for evaluation of a functional murmur detected twenty years earlier the presence of which had caused the patient to lead upon doctor's advice a sedentary life ever since.

The selection of control subjects ought to give a wide range for the normal variations of anthropometric and circulatory variables in the present age group No obvious overrepresentation of subjects with unduly large circulatory systems that significantly could influence the results of comparisons between patients and control group should be present.

IV

METHODS

A. Laboratory investigations

The total amou t of hemoglobin

Determination of the total amount of hemoglobin was mad with the alveolar carbon monoxide method (Sjöstrand 1948) modified by Wiklander (1956) The stand ard error of a si gle determination cal culated from duplicate determinations was 4 ☐

The heart olumes

Roentgenological estimation of the heart volume was done with the patient both in the standing and the recumbent position. The standing heart volume determination was done according to Lysholm (Liljestrand et al. 1939) Relative heart volume, e. ml m² BSA, was estimated according to Jonsell (1939) Total heart olume was estimated from roentgenograms taken with the patient in the prone position and with exposures in two planes without special re gard to the contraction phases f the heart according to Kjellberg et al. (1949) The posterio anterior projections were however not taken with a 30° angled tube and the olumes were calculated according to Jonsell No analysis of the error of this method was made on the present material, but the error of the method at the laboratory ex pressed as the ariation coefficient of single determination was previously found to be 3 3 (Axén et al 1946)

The el ctrocard gram

The lectrocardiogram at rest was recorded with the subject in the supine position with a four channel direct writing ink jet electrocardiograph (Mingograph 42, Elema Co) The following leads were used at rest I, II, III, aV_R aV_L aV_F CR_1 CR_2, CR_4 CR_5 CR_7 and V_1 V_2, V_4 V_5, V_7 During exercise the reference electrode was placed on the forehead and CH_2, CH_4 CH_5 and CH_7 were recorded. After exercise lead I, II, III, CR_1 CR_2, CR_4 CR_5 CR_7 were recorded. Holmgren and Strandell (1961) have shown that CR and CH leads are comparable when used in this type of test. The ECG:s were interpreted according to Lepeschkin (1960) and Mattingly (1962) The coding system introduced by Blackburn et al. (1960) modified by the Research Committee of the International Society of Cardiology in 1963 was used. Special attention was paid to the evidence of myocardial scarring (abnormal initial vectors) ischemia (ST and T vector abnormalities) or ventri cular arrhythmias when the results were analyzed.

In the analysis only Q-waves class I 1 and 2, and ST J depressions of 1 mm or more (measured from the P R-segment at the beginning of the QRS complex) and horizontal or downward sloping ST-segments were considered abnormal. Lesser Q waves and T changes (code I 3 and V 5) and isolated J depressions were considered insignificant and disregarded. Exercise and post exercise ECG:s were coded using the resting ST-segment as reference level. (Fig. 1)

40—60 years of age fulfilling all criteria outlined above.

In spite of the strict criteria the present group of subjects was heterogenous in several respects. The number of previous clinically recognized infarctions varied between one and four among the patients with a diagnosis of myocardial infarction Among the nineteen patients with a diagnosis of only angina pectoris, six were found to have initial QRS vector deflections suggestive of previous clinically silent myocardial infarctions Therefore the division of the patients into these two groups was rather arbitrary There was also considerable variation in the duration of symptoms ranging from about 6 months in some cases to more than 10 years in three cases.

The association between coronary heart disease and hypertensive cardiovascular disease is well established (Biörck et al. 1958) (Wahlberg 1963) It is also often noted that a permanent arterial hypertension disappears after a myocardial infarction The present material was probably heterogenous as to the presence of previous hypertension. As it was impossible to evaluate this factor it was instead minimized by exclusion of all patients with electrocardiographic signs of left ventricular hypertrophy and fundus hypertonicus III—IV

It is important that a control group used for comparison with patients does not include only subjects with large circulatory capacities and consequently supernormal hemodynamic variables. The subject s physical fitness is therefore of importance for the selection of a suitable control group The present control group consisted of men of varying body size with different degrees of daily physical activity and with physical working capacities varying between 600 and 1500 kpm/min The volunteers were from fire brigades, but only two (no 41 and 45) of them were still on active duty as firemen and thus perhaps more physically fit than the rest of the group The least physically fit in the control group was a man (no 39) referred for evaluation of a functional murmur detected twenty years earlier the presence of which had caused the patient to lead upon doctor s advice a sedentary life ever since

The selection of control subjects ought to give a wide range for the normal variations of anthropometric and circulatory variables in the present age group No obvious overrepresentation of subjects with unduly large circulatory systems, that significantly could influence the results of comparisons between patients and control group should be present

IV

METHODS

A. Laboratory investigations

The total amount of hemoglobin

Determination of the total amount of hemoglobin was made with the alveolar carbon monoxide method (Sjöstrand 1948) modified by Wiklander (1956) The standard error of single determination calculated from duplicate determinations was 4 %

The heart volumes

Roentgenological estimation of the heart volume was done with the patient both in the standing and the recumbent position. The standing heart volume determination was done according to Lysholm (Liljestrand et al 1939) Relative heart volume i.e. ml m² BSA, was estimated according to Jonsell (1939) Total heart volume was estimated from roentgenograms taken with the patient in the prone position and with exposures in two planes without special regard to the contraction phases of the heart according to Kjellberg et al (1949) The postero-anterior projections were however not taken with 30° angled tube and the volumes were calculated according to Jonsell. No analysis of the error of this method was made on the present material, but the error of the method at the laboratory expressed as the variation coefficient for a single determination was previously found to be 3.3 (Axén et al. 1956)

The electrocardiogram

The electrocardiogram at rest was recorded with the subject in the supine position with a four channel direct writing ink jet electrocardiograph (Mingograph 42, Elema Co) The following leads were used at rest I, II, III, aV_R aV_L V_F CR_1 CR_2, CR_4 CR_6, CR_7 and V_1 V_2, V_4 V_5, V_7 During exercise the reference electrode was placed on the forehead and CH_2, CH_4 CH_5 and CH_7 were recorded. After exercise lead I II III, CR_1 CR_2, CR_4 CR_5 CR_7 were recorded. Holmgren and Strandell (1961) have shown that CR and CH leads are comparable when used in this type of test. The ECG:s were interpreted according to Lepeschkin (1960) and Mattingly (1962) The coding system introduced by Blackburn et al. (1960) modified by the Research Committee of the International Society of Cardiology in 1963 was used. Special attention was paid to the evidence of myocardial scarring (abnormal initial vectors) ischemia (ST and T vector abnormalities) or ventricular arrhythmias when the results were analyzed

In the analysis only Q-waves class I 1 and 2, and ST J depressions of 1 mm or more (measured from the P R segment at the beginning of the QRS complex) and horizontal or downward sloping ST-segments were considered abnormal Lesser Q waves and T changes (code I 3 and V 3) and isolated J depressions were considered insignificant and disregarded. Exercise and post exercise ECG:s were coded using the resting ST-segment as reference level (Fig 1)

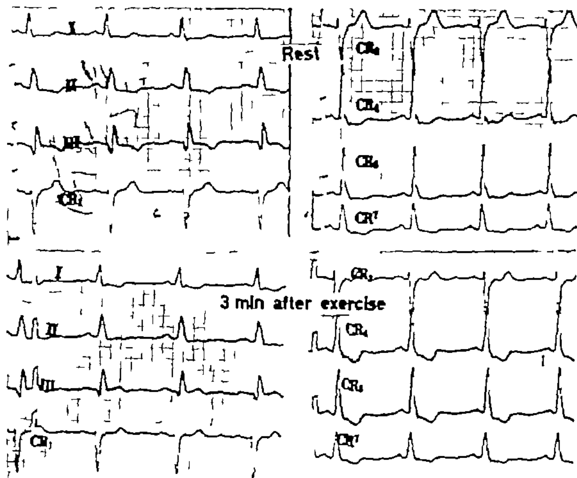


Fig 1 Significant initial QRS vector abnormality and ST depressions at rest with accentuated ST depressions and T wave inversion 3 minutes after exercise (case no 11)

The ECG s were coded independently by the author and one experienced electrocardiographer Irma Astrand, M D with no significant disagreement.

The exercise tolerance test

The exercise tests were done on a bicycle ergometer in the sitting position described by Holmgren and Mattsson (1954) Nitroglycerin or other vasodilators were in all cases withdrawn at least 24 hours before the tests. The exercise tests were usually done with the patients in the semifasting state in the afternoon The pedalling rate was 60 rpm, and the initial work load varied between 150—300 kpm/min The work load was increased in steps of the same magnitude as the initial work load each 6th minute as proposed by Sjöstrand (1947) and Wahlund (1948)

Before starting the exercise test the purpose of the procedure was explained. The subject was instructed to continue the test until his symptoms (angina pectoris, dyspnea, fatigue) became so severe, that he wished to discontinue the exercise In such a fashion an individual exercise tolerance limited by subjective symptoms was estimated in each case

If the patient discontinued the test after less than 2 minutes on a given work level, his exercise tolerance was estimated to be the previous work level. When able to continue more than 2, but less than 4 minutes, the patient's exercise tolerance was estimated to be the arithmetic average of the highest work load experienced. Patients able to continue more than 4, but less than 6 minutes on a given work level were estimated to have that work load as their exercise tolerance.

B. Hemodynamic Investigations

The cardiac catheterization

Right heart catheterization was performed in essence according to the method originally described by Cournand and Ranges (1941). Studies were performed in the morning with the subject in the postabsorptive state, premedicated with 0.1—0.15 sodium amytal per os, given one hour before the start of the examination.

Patients and hospitalized control subjects were brought to the laboratory in bed, while the volunteers were ambulatory and were allowed 30 minutes rest on the examination table before the procedure was started.

A double-lumen catheter (no. 8 or 9) was introduced through an incision on the antecubital vein of the left arm. A single lumen catheter (no. 9) was used in three cases, where double lumen catheters could not be passed into the superior vena cava, due to venous spasm in the axillary region. Using the percutaneous technique of Seldinger (1953) a thin 35—45 cm long teflon catheter was introduced about 10—15 cm into the right brachial artery. In four cases an 100 cm long catheter with a flexible stainless steel wire guide was used in order to allow retrograde catheterization of the left ventricle. The venous catheter tip was, with the aid of fluoroscopy, manipulated into the main pulmonary artery and pressures from the tip and the side hole, located in the right ventricular cavity, were recorded.

Expired air was collected in a Douglas bag during 5 minutes after an initial 2 minute adaptation period. After 2½ minutes, simultaneous blood samples were drawn during 30 seconds from the main pulmonary artery and the brachial artery. The tip of the catheter was then manipulated into the "wedge" position in the peripheral pulmonary vasculature, and the pressures from the pulmonary artery and the pulmonary capillaries were simultaneously visualized on an oscilloscope and recorded.

A bicycle ergometer adapted for exercise in the supine position was attached to the examination table, and the subject's feet were strapped to the pedals. Before the exercise test was begun the patient was instructed to signal with his head during the air collection, should such severe subjective symptoms of angina or dyspnea develop, that he wished to discontinue the exercise period before the study was completed.

A suitable work load (usually 250 kpm/min.) was selected and an exercise period of about 8—10 minutes was started. Initial exercise pressures were usually recorded after 2 minutes at which time the collection of expired air was also started. The heart rate and simultaneous samples of arterial and

mixed venous blood were obtained after 4½ minutes of exercise, prior to and after which the pressures were recorded. If at the end of the air collection period, the subject was asymptomatic and considered able to continue the exercise test, the work load on the ergometer was increased and a new exercise period was started In the majority of cases, after 5—6 minutes of exercise, the tip of the catheter was withdrawn from the wedge position to the main pulmonary artery so that the side hole became located in the right ventricular cavity whereupon these pressures were recorded.

In twenty-one cases a slow intravenous injection of 1.2—1.6 mg of lanatoside C (Cedilanid ® Sandoz AG) was given a few minutes after the exercise study was completed, whereas in nine cases this was substituted by saline solution These patients were then allowed to rest on the table under constant supervision for approximately one hour after which the whole rest and exercise procedures were repeated.

Complications In one patient, marked anxiety already present during the rest period was so accentuated by cardiac catheterization that further studies were prevented One other patient developed a pericardial effusion with signs of cardiac tamponade during the exercise period. Prompt discontinuance of exercise and removal of 25 ml of blood by pericardial puncture led to a rapid recovery The cardiac catheterization results in these two cases were not included in the present study

One other patient developed a substantial antebrachial and pectoral hematoma four hours after completion of the investigation. It was thought to be due to an extrathoracic rupture of the subclavian artery of the right arm, induced by the retrograde arterial catheterization. Vascular surgery was not required and the hematoma was absorbed after one week, leaving no pulse diminution or other residual.

Pressure curves from the right and left ventricles, pulmonary and brachial arteries were obtained with strain gauge manometers on a six channel photographic oscillograph (Elema Co) or an eight channel direct writing ink jet oscillograph (Mingograph 81 Elema Co) Mean pressures were obtained with electrical integration. *Zero level* for the strain gauges during the investigation was the mid thoracic line measured at the sternal insertion of the fourth rib The electrical standards of the manometers were calibrated in connection with each investigation using a hydrostatic standard corresponding to a pressure of 50 mm of Hg above the zero line The Fick principle method for determination of the *cardiac output* was employed. Expired air collected in Douglas bags during 5 minute periods was analyzed in duplicate with the Haldane technique in order to obtain the amount of oxygen consumed per minute Arterial and mixed venous blood samples for determination of the a-vO_2 difference, drawn simultaneously during the air collection were analyzed in duplicate for hemoglobin content, oxygen saturation and content with a Beckman B spectrophotometer according to the methods described by Drabkin (1945) and Nahas (1951) as modified by Holmgren and Pernow (1959) No analysis of the errors of these methods with duplicate determination was made on the present material but variation coefficients of single determinations with this technique previously established by Holmgren and Pernow (1960 A) were

for the oxygen uptake 3.6 % at rest, 4.3 % during exercise

for the a- O_2 difference 5.4 % at rest, 3 % during exercise

for the cardiac output 8.2 % at rest, 5.2 % during exercise.

Heart rates were recorded during a minimum of 20 seconds. The *stroke volume* was obtained by dividing the cardiac output with the heart rate measured with ECG during the blood sampling.

The vascular resistance was calculated using Poiseuill's law (Wiggers 195) and was expressed in cgs units. The pulmonary vascular resistance expressed in dyn. sec. cm−5 was calculated as the difference between the pulmonary artery mean and pulmonary capillary venous mean pressures (wedge) in mm Hg divided by the cardiac output in ml/sec. and multiplied by 1332. The systemic vascular resistance expressed in dyn. sec.cm−5 was calculated as the arterial mean blood pressure in mm Hg divided by the cardiac output in ml/sec. and multiplied by 1332.

C. Statistical methods

Conventional statistical methods were used for calculation of the arithmetic mean (M) the standard error of the mean (S.E.) the standard deviation of the mean (S.D.) and the variation coefficient of a single determination (C). Significance of differences between mean values were tested by Student's t test.

The degree of probability was denoted as

probably significant $= 0.05 > P > 0.01$
significant $= 0.01 > P > 0.001$
highly significant $= 0.001 > P$

Wilcoxon's rank sum test for two samples was used for testing the significance limits of differences in the clinical-hemodynamic correlations (Snedecor 1957). Conventional methods were used for analysis of regression and correlation (Dixon and Massey 1957).

D. Comments

The laboratory methods employed in the present study are all well established and generally accepted for clinical application. Some of the methods and their application on the material deserve, however, to be commented upon.

An exercise tolerance test was done on all patients regardless of whether the resting electrocardiogram was normal or abnormal, and the test was usually terminated not because of ECG changes but because of angina pectoris. Performed in this manner the tests quantitatively measure the pain threshold for each individual, as influenced by varying individual motivation and pain sensitivity and is thus not a completely objective parameter. In spite of this the reproducibility of the results have been found to be acceptable (Kinsella et al. 1962) (Hallén 1964). One definite advantage with this type of exercise test is that the results with reasonable degree of accuracy give

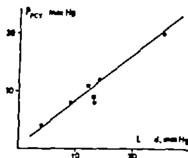


Fig 2 The relation between simultaneously measured PCV mean and left ventricular end-diastolic pressures at rest and during exercise in 4 cases. Circles denote cases with myocardial infarction, squares control subjects. Open symbols denote values at rest, filled symbols values during exercise. Regression equation for the regression line: $\bar{P}_{PCV}=0.69+0.79\ LV_{ed}$ (r=0.83, S.D.=±2.9 n=11)

an indication of what amount of physical work the patients can tolerate in ordinary daily life.

The hemodynamic studies were done with the ordinary right heart catheterization technique with cardiac output determinations using the Fick principle. This technique was in the beginning of the study combined with retrograde left ventricular catheterization from the right brachial artery in a small number of cases for the purpose of obtaining left ventricular end-diastolic pressures both at rest and during exercise. Undamped left ventricular end-diastolic pressures were obtained in four cases at rest and during exercise. These left heart catheterizations were discontinued because of the previously mentioned complication and difficulties locating the catheter tip in the left ventricular cavity and keeping it in place during exercise. However the correlation between the simultaneously measured PCV mean pressures ($\bar{P}_{PCV}$) and the left ventricular end-diastolic pressures (LV_{ed}) was good ($\bar{P}_{PCV}=0.69+0.79\times LV_{ed}$, $P<0.01$ r= 0.83 $P<0.01$) (Fig. 2) and thus the $\bar{P}_{PCV}$ could be used as an indicator of the end diastolic pressure changes in the left ventricle (Braunwald and Frahm 1961 A)

CORONARY ANGIOGRAPHY

by R. O. Malmborg, B. Nordenström and G. Törnell

Method

The method of coronary angiography used in the present study was a clinical application of Boerema's and Blickman's method (1955) modified by Nordenström (1960).

This method employs elevation of the intrabronchial pressure to obstruct inflow to the right side of the heart. Decreased outflow of blood from the left ventricle is thus obtained so that dilution of the contrast medium simultaneously injected through a catheter located in the ascending aorta is minimized. The higher specific gravity of the contrast medium, as compared to that of blood, is utilized for directing the contrast to the coronary arteries and to induce layering of the medium in the vessels.

Procedure

Under general anesthesia achieved by intravenous injection of sodium pentobarbital the trachea was intubated and the voluntary muscles relaxed by means of intravenous Celocurine® (Astra) administration. With percutaneous technique (Seldinger 1953) an Ödman-Ledin catheter was introduced through one of the femoral arteries and the tip placed in the ascending aorta immediately above the aortic valves. In the same femoral artery a thin polythene catheter was introduced for continuous recording of the arterial blood pressure. The patient was placed in 45° oblique prone position on a rapid biplane rollfilm changer (Gidlund Elema) with the left anterior chest against the film, so that the coronary arteries were positioned lower than the ascending aorta and on the same horizontal level. The intrabronchial pressure was raised to 50 cm of water by means of a specially developed oxygen injector and the blood pressure recording monitored continously on an oscilloscope. When the systolic arterial blood pressure was lowered about 50 % to a level of 50—60 mm Hg the medium (1 ml Urografin 76 % per kg body-weight) was injected within approximately 3 seconds into the ascending aorta. Exposures were made with a frequency of six frames/sec. during 4 seconds and then one per sec. during 10 seconds and the intrabronchial positive pressure was released. Radiographs were obtained during two separate contrast injections in two square angle planes. Slight, transient QRS and ST-T changes as well as occasional VEB:s were sometimes noted in the ECG during passage of the contrast medium through the coronary vessels and the myocardium.

Complications

In general, coronary angiography using the present method, is well tolerated even by patients with advanced coronary heart disease In more than 350 coronary angiographies on patients mainly suffering from coronary insufficiency there has been no fatality with the investigation. However one fatality due to irreversable ventricular fibrillation already during the induction of the anesthesia occurred in one case

In the present material complications occurred in only one case in which a slight leftsided hemiparesis was present 4 hours after the examination but completely disappeared after 48 hours.

Interpretation

Interpretation and evaluation of the coronary angiographies were made by one of the roentgenologists having no knowledge of the case histories or the clinical data of the patients

The atherosclerotic vascular changes observed on the angiograms were graded according to the following classification

0 No atherosclerotic changes visible

Grade I Atherosclerotic changes with narrowing of less than half the arterial lumen.

Grade II Atherosclerotic changes with narrowing of more than half the arterial lumen or down to 1 mm in diameter

Grade III Total occlusion of the vessel

It was not considered possible to evaluate and classify the collateral circulation often noted in cases with total occlusion.

The present method with serial radiographs in two different projections usually provided good visualization of the three main coronary arteries the right coronary artery the left anterior descending branch and the left circumflex branch of the left coronary artery Thus, it was possible to evaluate all three branches in twenty-eight of thirty-one cases In the remaining three cases only two branches could be evaluated due to technical errors during one of the two injections.

The changes in the three main coronary arteries were evaluated and graded separately in every case giving every patient three different figures in the angiographic evaluation, theoretically ranging from minimally 0 0 0 to maximally III III, III

Results

Coronary angiographies were done in thirty-one of the thirty-eight cases included in this study The individual results classified according to the grading system are presented in table I.

All patients examined had significant angiographic evidence of atherosclerosis in at least one of the major coronary vessels One patient had occlusions in all three coronary arteries (Fig I) whereas the

Table I. Primary coronary angiographic data for 31 male patients with coronary heart disease.

Case No.	Clinical diagnosis	Coronary artery changes Grade I—III		
		Left anterior cor art.	Lef circumflex cor art.	Right cor art.
1	St p.inf.myoc.	III	II	III
3	St.p.inf.myoc.	III	0	I
4	St p.inf.myoc.		III	II
5	St.p.inf.myoc.	II	I	III
7	Ang. pect.		I	III
8	Ang. pect.	III	III	III
10	Ang. pect.	III	II	II
12	Ang. pect.	III	II	III
13	St.p inf.myoc.	III	I	I
14	Ang. pect.	III	I	I
15	Ang. pect.	II	0	I
16	Ang. pect.	III	II	0
17	St p.inf.myoc.	I	III	III
19	St.p.inf.myoc.	II	I	III
20	St.p inf.myoc.	II	0	I
21	St.p.inf.myoc.	II	I	III
22	Ang. pect.	III	II	I
23	St.p.inf.myoc	III	I	II
24	St p.inf.myoc.	II	0	0
25	Ang. pect.	I	II	III
26	St p.inf.myoc.	I	I	I
27	Ang. pect.	II	III	II
28	Ang. pect.	II	I	III
29	Ang. pect.	III	III	II
30	Ang. pect.	II	III	III
31	Ang. pect.	III	II	III
32	Ang. pect.	III		I
33	St p.inf.myoc.	III	I	III
34	Ang. pect.	III	II	I
35	Ang. pect.	III	I	II
38	Ang. pect.	II	0	I

mildest had only Grade I changes in all three arteries. Only seven coronary arteries among six patients were free of angiographic changes indicative of atherosclerosis (Fig. II). Grade I changes were found in twenty four arteries in eighteen cases (Fig. II) Grade II in twenty-four arteries in twenty one cases (Fig. III) and Grade III in thirty five arteries in altogether twenty-six cases (Fig. I and II). The distribution of the

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A B

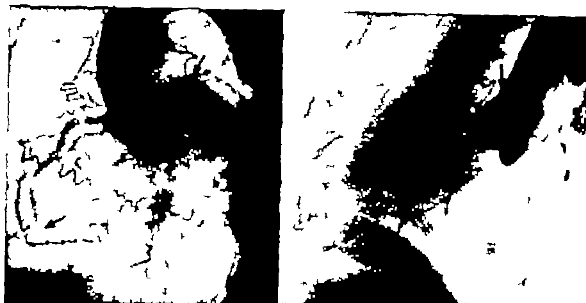
Fig. III Case no 35. Fifty year old man with ten year history of angina pectoris but no known myocardial infarction. Functional Class II. Normal QRS complex and ST-segment in the ECG at rest, ST depressions appearing during exercise. Coronary angiography: *A) Severe narrowings of the right* coronary artery (Grade II). Atherosclerotic change with narrowing of less than half the lumen of the *left circumflex branch (Grade I). B) Occlusion of the left anterior descending branch one cm from* the origin (Grade III). Retrograde filling via collaterals in the apical region.

Table II The distribution of the atherosclerotic changes in the three main coronary artery branches in 31 male patients ith coronary heart disease

Coronary artery changes	Lef anterior coronary artery	Left circumflex coronary artery	Right coronary rtery	Total number
No hanges	0	5	2	7
Grade I	3	11	10	24
Grade II	10	8	6	24
Grade III	16	6	13	35

Tabl III The relation between clinical diagnoses and the coronary artery changes in 31 male patients ith coronary heart disease.

		Coronary artery changes			
Clinical diagnosis	Number of cases	N hanges	Grade I	Grade II	G ade III
St.post inf myoc.	13	4	13	8	13
Angina pectoris	18	3	11	16	22



A B

Fig. I Case no. 8 *Fifty-five year old* man with a history of angina pectoris for more than ten years but no known myocardial infarction. Functional Class III Normal ECG at rest. ST depression during and after exercise Coronary angiography: Occlusion of all three main branches of the coronary arteries A) Distal occlusion of the right and proximal occlusion of the left circumflex branch. B) *Proximal occlusion* of the anterior descending branch. Distal filling through collaterals from the right coronary artery



A B

Fig. II Case no F fty-one year ld man with a history of one acute myocardial infarction eight months prior to th tudy and angina pectoris for eighteen months. Functional Class II. Normal ECG at rest, during or fter exercise Coronary angiography: A) Slight narrowing of the right coronary artery 4 cm below the origin (Grade I) Occlusion of the left anterior descending branch at the origin, w th retrograde filling of the artery via collaterals in the apical region (Grade *III*) No s-ible changes in the left circumflex branch. B) Contrast medium remaining in the proximally occluded descending branch when other main branches are emptied.

There are at present no premeditated studies of coronary angiography in clinically healthy individuals of similar or other age groups available for comparison. Littman and Forsberg et al. found fewer changes at coronary angiography in patients with symptoms and ECG contradicting coronary heart disease, than in those with typical symptoms and pathological ECG. The present material was too selected and too small to permit any conclusions from comparison between clinical diagnosis, ECG, functional capacity or exercise tolerance on the one hand and the graded coronary angiographic changes on the other. When more experience has been obtained from larger series of cases, also including patients with mild to moderate and early symptoms of coronary insufficiency, such comparisons can be done and might then prove themselves to be of value for diagnostic and prognostic purposes.

Conclusions

The coronary angiographies in thirty-one of the thirty-eight patients in this study all contained significant changes in the three main coronary arteries indicative of atherosclerosis. With the present grading system there could be no difference demonstrated between patients with and without myocardial infarctions, normal and pathological ECG, good and poor functional capacity and normal and low exercise tolerance.

changes in the different arteries is demonstrated in table II

There was no difference in severity of changes between patients *with* and those *without previous known infarction* as illustrated in table III The same lack of difference was also noted when patients with significant ECG *abnormality at rest* Q waves or ST depressions, were compared with those without, as illustrated in table IV The angiographic changes were also compared with the individual *functional capacities* and it was then evident that coronary heart disease patients with mild symptoms (Functional Class I—II) may have severe angiographic changes with one or several occlusions. A comparison between the coronary angiographies and the *exercise tolerance* revealed no significant relation between the degree of changes or number of occlusions and the exercise tolerance.

Table IV The relation between ECG changes at rest and the coronary artery changes in 31 male patients with coronary heart disease.

		Coronary artery changes			
ECG changes at rest	Number of cases	No changes	Grade I	Grad II	Grade III
Normal QRS compl.	19	6	12	16	22
Significant Q-wave	12	1	12	8	13
Normal ST segm.	21	5	14	18	23
Significant ST depr	10	2	10	6	12

Comments

The present method for grading the coronary angiographies was introduced mainly in order to facilitate the recording of the results in tables and figures The grading system does not take into account the location of the obstructions, the absence or presence of a collateral circulation in cases with occlusions, marked narrowing of the coronary arteries, or the appearance of the capillary phase. It can consequently not be used for accurate evaluation of the perfusion of the different myocardial segments supplied by the three main coronary arteries, but only to indicate the absence or presence of more or less significant atherosclerotic changes in the arteries.

Reports on the correlation between the clinical symptoms (Littman 1961 Forsberg et al. 1963) electrocardiographic changes (Messer et al. 1961 Forsberg et al. 1963, Kattus et al. 1963) findings at surgery (Sloman and Hare 1960 Hallén 1964) and autopsies (Gray et al. 1962, Niles and Dotter 1963) on one hand, and findings at different forms of coronary radiography on the other are at present available In all these studies was concluded that a positive coronary angiogram indicative of obstructive disease with few exceptions was associated with clinical symptoms and signs of coronary heart disease. The findings in the present study fully supported earlier reports

There was no significant difference in the relation between THb and body surface area for the control subjects and the patients, nor between those with and without previously known infarctions.

Heart volumes

In all patients the hearts were normally shaped without roentgenological evidence of either isolated or combined atrial or ventricular enlargement.

Relative heart volumes in the standing position varied for the patients between 300—530 ml/m² BSA with a mean of 420 (S D = ±60) ml/m² BSA. The mean relative heart volume for patients with previous infarctions was 425 (S.D = ±67) ml/m² BSA and for those without 415 (S D = ±52) ml/m² BSA. The differences between these mean values and the mean value for the control material were not significant.

Total heart volumes in the recumbent position were measured in thirty-one cases and varied between 610 and 1150 ml with a mean volume of 848 (S.D = ±144) ml. Patients with previous infarctions had volumes varying between 610 and 1110 ml with a mean of 866 (S D = ±164) ml, and those without known infarction volumes varying between 680 and 1150 ml with a mean of 829 (S D = ±120) ml. The difference between the mean total heart volume for all patients combined and the mean total heart volume for the control subjects was significant ($P<0.01$). The difference between mean total heart volumes for patients with or without previous infarction was not significant.

In the control subjects the relative heart volumes varied between 320 and 500 ml/m² BSA with a mean of 439 (S D = ±49) ml/m² BSA.

Total heart volumes were found to vary between 850 and 1070 ml with a mean of 929 (S.D = ±70) ml.

Comments Since the present study was aimed at investigating central hemodynamics in patients with coronary heart disease *without clinical evidence of congestive heart failure* patients with obvious cardiac enlargement were excluded. Thus, only patients with relative heart volumes ≤530 ml/m² were accepted for the study. This limit was based upon data collected by Garlind and Lindgren (1958) in a study of 3000 clinically healthy males in different age groups seeking municipal employment in the city of Stockholm. In that material heart volumes, both absolute and relative, increased with age, and also varied with the amount of physical activity during ordinary daily life. The mean relative heart volume calculated by Lindgren and Odén (1954) for subjects in Garlind's material between 40 and 49 years of age was 416 ml/m² BSA (S.D = ±56) and for subjects between 50 and 59 years of age 419 ml/m² BSA (S.D = ±55).

When the individual total heart volumes were related to the total hemoglobins, the majority of the cases included in this study had volumes within the range observed by Strandell (1964, C) in clinically healthy men, as illustrated in fig. 4. The regression line for this relation was based upon heart volumes obtained with a somewhat different technique than employed in the present study accounted for under Methods. The difference in technique can however be compensated for by the use of a regression equation, calculated by Holmgren (personal communication 1964) which is based upon the results of heart volume estimations with both techniques on seventeen healthy sub-

VI

RESULTS

A. Laboratory Investigations

Total amount of hemoglobin

All *patients* had hemoglobin concentration above 12 g/100 ml. The total amount of hemoglobin for the thirty-eight patients varied between 500 and 965 g with a mean amount of 718 (S.D = ± 125) g

For the nineteen patients with previous infarctions the THb varied between 535 and 965 g, with a mean of 728 (S.D = ± 134) g Nineteen patients without known infarctions had THb:s varying between 500 and 880 g with a mean of 708 (S.D = ± 119) g

No significant differences could be demonstrated between the mean values for all patients combined or separated after clinical diagnosis and the mean value for the control subjects

The *control subjects* all had hemoglobin concentrations above 12 g/100 ml. The total amount of hemoglobin varied between 620 and 920 g, with a mean amount of 749 (S D = ± 94) g

Comments The individual THb:s can be related to body surface area (calculated from height and weight) and compared with the regression line for this relation among healthy males between the age of 30 to 83 years, established by Strandell (1964, D) as illustrated in fig. 3 It is seen in this figure that three of ten control subjects had lower THb:s than what could be predicted from their body surface area, when compared with the regression equation ± 2 S D In two of the subjects the THb values were within ± 3 S.D for the predicted values (case no. 47 49) whereas in the remaining case (case no 40) the difference was more marked Among the patients there were ten subjects who had THB:s that fell outside of ± 2 S.D for the regression between THb and body surface area. In five of these cases the values fell within ± 3 S.D for the predicted values whereas in the remaining five cases the difference was more marked

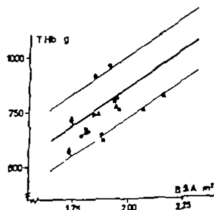


Fig 3 Total hemoglobin in relation to body surface area in 38 patients and 10 control subjects. The regression line ± 2 S.D represents the interindividual variation for 74 healthy males 30—83 years of age (Strandell 1964, D) Circles denote patients with myocardial infarction, triangles patients with angina pectoris only and filled squares control subjects.

ventricular ectopic beats. One patient had ventricular ectopic beats both at rest and during work. In three cases the ectopic beats were the only added abnormality as compared to the resting ECG. Seventeen of twenty six patients with normal ST-segments at rest developed significant ST depressions during exercise. In ten of twelve patients with "ischemic" ST depression at rest, these changes were significantly accentuated during exercise (increase of 1 mm or more below resting level). Isolated T wave changes occurred in six cases, including two patients in whom inverted T waves became positive during work.

Three minutes after exercise ventricular ectopic beats were present in two patients, in one of them being the only added abnormality when compared with the ECG at rest. Sixteen of twenty six patients with normal ST-segments at rest had significant ST depressions 3 minutes after exercise. Thirteen of these developed ST depressions already during exercise. In eight of twelve patients with ST depressions already present at rest these changes were significantly accentuated after work. Four patients had significant T wave changes without ST changes after work.

The ECG's of the *control subjects* were all considered to be normal at rest, during and 3 minutes after exercise.

Comment. The high specificity and diagnostic ability of the present coding system for myocardial infarction has been established in both clinical and anatomical comparative studies by Blackburn *et al.* (1960). In a comparative ECG and necropsy study Mattingly (1962) reported a high diagnostic significance and good correlation of ischemic ST changes with coronary heart disease but poor correlation for T wave changes in the postexercise electrocardiogram. The results of the present ECG study in a selected group of patients with coronary heart disease are in accordance with the results of Blackburn, Mattingly and others. Out of nineteen patients previously hospitalized for myocardial infarction, twelve had significant initial QRS vector abnormalities. Six had normal QRS complexes but significant ST depressions at rest or 3 minutes after exercise and only one had completely normal ECG's. The lack of QRS abnormalities during and after known myocardial infarctions have previously been reported by, among others, Blackburn (1960) and Pappas (1958).

In nineteen patients with a clinical diagnosis of coronary insufficiency (angina pectoris) but no clinically diagnosed previous infarction, "Q waves" were found in six patients and "ischemic" ST changes at rest or after exercise in the remaining thirteen cases. Similar findings have previously been reported from the Framingham studies, where at least 20 % of the seventy-three patients with electrocardiographic evidence of myocardial infarction were clinically unrecognized (Stokes and Dawber 1959).

The clinical diagnosis of coronary heart disease was supported by electrocardiographic signs at rest, during and/or after exercise in thirty-seven of the studied cases. The one patient (case no. 3) in whom ECG changes were lacking had a myocardial infarction with anteroseptal ECG changes 8 months prior to the study and had also subjective symptoms of coronary insufficiency consisting of angina pectoris and dyspnea upon effort. At the time of the study no subjective symptoms suggestive of coronary insufficiency could, however, be precipitated during the ordinary exercise tolerance test.

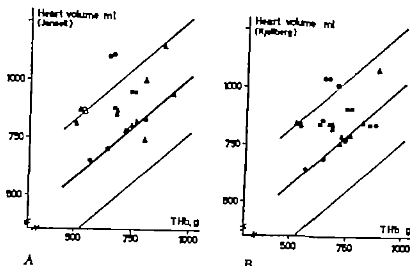


Fig 4 Total heart volume A) observed and B) converted in relation to total hemoglobin in 31 patients and 10 control subjects. The regression lines ± 2 S.D represent the interindividual variations for 74 healthy males aged 30—83 years (Strandell 1964, C) Symbols as in fig. 3.

jects The equation reads H.V (Kjellberg) = H V (Jonsell) × 0 87 + 79 (r = 0 882 S.D = ± 80 8) From this equation it can be seen that, within the present range the volumes observed were somewhat larger than those that could be deducted from the conversion equation. Thus the heart volume/total hemoglobin relations for the majority of cases in the present material were essentially equal to those observed by Strandell among healthy males between 30 and 83 years of age as illustrated in fig 4 B Six of the subjects (five patients and one control subject) (case no 1 3 5 17 29 47) had observed or converted heart volumes larger than 1000 ml When these volumes were related to the individual body weight three patients (case no 1 5 17) had volumes that fell outside the 95 % confidence limits for the heart volume/body weight relation among healthy males. None of them were obese. When related to the individual total hemoglobin the six patients with volumes larger than 1000 ml had volumes that in five cases fell outside the 95 % confidence limits for this relation among healthy men. In two of the cases (case no 3 47) this could be explained by low total hemoglobins that were out of proportion to their body surface area as illustrated previously (Fig 3)

The observed heart volumes were also compared with predicted volumes deducted from multiple regression equations established by Strandell with body weight and age as independent variables. When these factors were taken into account no significant differences could be noted between the observed and predicted heart volumes for either control subjects, patients with or those without previously known infarctions.

ECG analysis

The results of the ECG analysis are summarized in table 3 (see Appendix)

At rest twelve out of thirty-eight *patients had normal ECG:s.* Occasional ventricular ectopic beats were recorded in two cases. Significant initial QRS vector abnormalities were found in eighteen cases ST depressions with or without T wave inversions of the ischemic type were present in twelve cases. Inverted T waves were the only abnormality in three cases.

During exercise six patients developed

ency comparable to those encountered by the patients during ordinary daily activities.

In thirty-four of the thirty-eight studied *patients* definite subjective symptoms of coronary insufficiency consisting of retrosternal chest pain was precipitated during the exercise tolerance test. Three patients experienced only general fatigue and therefore felt unable to continue the test. One test was discontinued because of ventricular arrhythmia. Combined dyspnea and angina pectoris was present in six cases.

The exercise tolerances varied between 100 and 900 kpm/min. Mean exercise tolerance for the whole group was 399 (S.D = ±201) kpm/min. The difference between the mean exercise tolerances for the patients and the control subjects was highly significant (P<0.001). The heart rate when symptoms occurred varied between 87 and 156 beats/min. with a mean of 121 (S.D = ±17) beats/min. The difference between mean heart rates for the patients and the control subjects was also highly significant (P<0.001).

For nineteen *patients with previous myocardial infarction* the exercise tolerance varied between 100 kpm/min. and 600 kpm/min. with a mean of 339 (S.D = ±178) kpm/min. The heart rates varied between 87 beats/min. and 147 beats/min. with a mean of 119 (S.D = ±17) beats/min.

The remaining nineteen *patients without previous myocardial infarction* had exercise tolerances varying between 200 kpm/min. and 900 kpm/min. with a mean of 458 (S.D = ±209) kpm/min. The heart rates varied between 90 beats/min. and 156 beats/min. with a mean of 123 (S.D = ±18) beats/min.

The difference between mean exercise tolerances for patients with or without infarction and the difference between mean heart rates when symptoms occurred was not significant.

When the patients were grouped after *functional capacity* (N.Y. Heart Ass. 1955) the exercise tolerance for five patients in Class I varied between 400 and 600 kpm/min. with a mean of 540 (S.D = ±89) kpm/min. The heart rates varied between 105 and 143 beats/min. with a mean of 120 (S.D = ±14) beats/min.

For nineteen patients in Class II the exercise tolerance varied between 100 and 900 kpm/min. with a mean of 484 (S.D = ±206) kpm/min. The heart rates varied between 104 and 156 beats/min. with a mean of 129 (S.D = ±14) beats/min.

Fourteen patients in Class III—IV (one in IV) had exercise tolerances varying from 100 to 400 kpm/min. with mean of 232 (S.D = ±72) kpm/min. The heart rates varied between 87 and 147 beats/min. with a mean of 110 (S.D = ±18) beats/min. Fig. 5.

The difference between the mean values for exercise tolerance for Class II and III—IV was highly significant (P<0.001) and the difference between mean heart rates was significant (P<0.01).

The exercise tolerances for fifteen patients with a low degree of *previous physical activity* (Group I) varied between 100—600 kpm/min. with a mean of 323 (S.D = ±163) kpm/min. The heart rates varied between 90 and 147 beats/min. with mean of 118 (S.D = ±15) beats/min. Nine patients with a moderate degree of previous physical activity (Group II) had exercise tolerances varying between 200 and 800 kpm/min. with a mean of 489 (S.D = ±209) kpm/min. The heart rates varied

Master (1944) Björck (1946) Simonson (1956) Lepeschkin (1960) and Mattingly (1962) among others have all stressed the importance of the post-exercise electrocardiogram in the diagnosis of coronary insufficiency In these studies only subjects with normal ST segments at rest were studied ST depressions in the resting electrocardiogram can be due to other abnormalities than myocardial ischemia caused by atherosclerosis in the coronary arteries (Mattingly 1962) As shown by Söderholm et al (1962) patients with clinical evidence of coronary heart disease and ST depressions at rest are, however often noted to develop further ST T changes during and 3 minutes after exercise In the present study such changes have been considered significant and thought to be ischemic in origin

The importance of ECG recordings during and after exercise of stepwise increasing intensity has been emphasized by Sjöstrand (1951 1960) They allow the investigator to study the gradual appearance of electrocardiographic changes during attacks of angina pectoris induced by effort, and if so desired, to discontinue a test when arrhythmias marked changes in the ST T region or the intraventricular conduction occur In the present study it was mainly attempted to measure what amount of physical work produced subjective symptoms (angina pectoris) comparable to those encountered in ordinary daily life In contrast to the majority of previous studies the exercise tolerance tests have therefore not been discontinued because of ST T changes, often appearing early during the present tests.

Significant ST depressions during exercise were present in twenty seven cases (71 %) Twenty five of these patients had angina pectoris when the exercise was stopped, the remaining two were stopped because of fatigue or dyspnea. The electrocardiograms registered 3 minutes after exercise had significant ST changes in twenty four of the thirty-eight cases (63 %) When both exercise and post-exercise recordings were considered thirty of the thirty eight studied cases had significant ST changes of the ischemic type (79 %) The presently observed high incidence of objective signs of coronary disease is higher than figures previously reported by Ford and Hellerstein (1957) Russek (1957) and Söderholm et al. (1962) The discrepancy was, however readily explained by different methods of selection being in the present study more liberal than in the above mentioned reports, where exclusion of patients with recent angina pectoris or ST changes at rest was made Out of the thirty four patients who developed angina pectoris during the exercise tolerance test twenty five had significant ST depressions during the test (73 %) and twenty-three cases 3 minutes after the exercise was stopped (68 %) If both the ECG during and 3 minutes after exercise were analyzed for significant ST changes when compared with the resting ECG twenty-eight cases had ST depressions in the presence of angina pectoris (82 %) Of the remaining six, all had significant QRS abnormalities suggestive of previous myocardial infarctions, but only two had ST depressions at rest.

Exercise tolerance test

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ency compatable to those encountered by the patients during ordinary daily activities.

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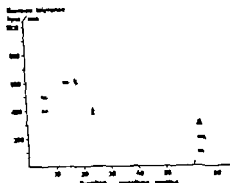


Fig. 6. Exercise tolerance in relation to duration of symptoms for 38 patients. Symbols as in fig. 3.

patients with angina pectoris as pointed out by Lundberg et al. (1961) Kinsella (1962) Hallén (1964) and others.

When subjective symptoms such as chest pain, dyspnea or general fatigue are used for measuring a parameter as in the present study the values obtained must be considered and evaluated with caution. Varying individual motivation, pain sensitivity and apprehension might to a varying extent influence the results. This method is, however the only presently feasible in a group of patients, with advanced and early appearing symptoms of coronary insufficiency In the present study the majority of the tests were discontinued at such low work loads and heart rates, that conclusions from steady state work on submaximal work loads regarding working capacity at any fixed heart rate, or maximal oxygen uptake as suggested by Sjöstrand (1951) and Åstrand (1952) respectively could not be drawn.

The individual day to day variation of the pain threshold in an exercise tolerance test among patients with coronary insufficiency was usually negligible, if the tests were performed under similar conditions as demonstrated by Kinsella and Hallén.

The exercise tolerances for the patients in the present material regardless of clinical diagnosis were in the majority of cases markedly lower than those of the control subjects and than those of other healthy men in similar age groups studied by Strandell (1964 B E) Strandell found a mean final work load for subjects between 40 and 49 years of age of 1004 (S.D = ±168) kpm/min., a mean heart rate of 172 (S.D = ±11) beats/min., for subjects between 50 and 59 years of age a mean of 945 (S.D = ±195) kpm/min. and a mean heart rate of 164 (S D = ±16) beats/min. These values correspond well with the values for the control subjects included in this study

There was no difference in exercise tolerance between patients *with or without previous myocardial infarction* indicating that the degree of subjective symptoms of coronary insufficiency was essentially identical for the two groups. A significant difference in exercise tolerance was noted between patients in *functional Class* II and Class III—IV in spite of the wide variation in exercise tolerance for the patients in Class II. The functional classification system does not take into consideration the varying requirements in physical activity (fitness) for different occupations and the duration of symptoms incapacitating the subjects. The wide variation in exercise tolerance in Class II can readily be explained by the differences in physical activity and duration of symptoms. When the patients were grouped according to their degree of previous physical activity (Figur 4) no significant differences in exercise tolerance could be noted between the three groups. However of the ten pa

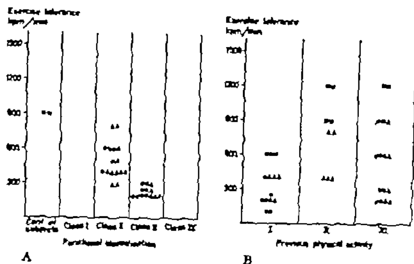


Fig 5 Exercise tolerance for 38 patients and 11 control subjects grouped after functional classification and previous physical activity. Symbols as in fig. 3

between 104 and 156 beats/min with a mean of 131 (S.D $= \pm 16$) beats/min. For fourteen patients with a high degree of previous physical activity (Group III) the exercise tolerance varied between 200 and 900 kpm/min. with a mean tolerance of 4.1 (S D $= \pm 215$) kpm/min. The heart rates varied between 87 and 14. beats/min with a mean of 117 (S D $= \pm 19$) beats/min Fig 5

The difference between the mean exercise tolerances and the mean heart rates for the different groups was not statistically significant

When the results of the exercise tolerance tests were compared with the *duration of symptoms* as illustrated in figure 6 there was no certain trend towards a reversed relation between exercise tolerance and duration of symptoms The higher exercise tolerances among the patients with previous infarctions were, however found only among those who had relatively short duration of their symptomatology whereas low exercise tolerances were found both in patients with short and long duration of symptoms

The difference between the mean exercise tolerance for twenty six patients with a duration of symptoms shorter than 5 years and for twelve patients with more than 5 years was not significant ($P > 0.05$)

All eleven *control subjects* were free of cardiac symptoms during and after the test. The tests were discontinued because of general fatigue or weakness in the legs. Limited by these subjective factors the exercise tolerances for the control group varied between 600 and 1500 kpm min. with a mean of 1036 (S.D $= \pm 246$) kpm/min. Fig 5

The heart rates at the end of the tests varied between 132 and 173 beats min. with a mean heart rate of 159 (S.D $= \pm 13$) beats/min.

Exercise tolerances and final heart rates for the patients are given in table 4 and for the control subjects in table 2 (see Appendix)

Comments Exercise tolerance tests using graded muscular work and simultaneous ECG recording can often be essential in properly estimating a patient's working capacity The test is also very useful when establishing the diagnosis of coronary disease as pointed out in the ECG analysis. The test can furthermore be used for evaluation of therapy in

studies were discontinued after one work period and one study after two work periods because of general caution or marked intracardiac pressure elevations without chest pain. The arterial oxygen saturations during exercise ranged for the patients between 100 % to 91 %. One of the control subjects had an arterial oxygen saturation during exercise of 88 % that could not be explained by any methodological error and in spite of normal routine pulmonary function tests and a normal chest x-ray.

During exercise the patients had higher heart rates, lower stroke volumes, higher intraventricular pressures and systemic resistances than the control subjects. The mean differences for these variables in patients and control subjects were all highly significant ($P<0.001$). The mean patient cardiac output was also lower than that of the control subjects ($P<0.05$). The significance of these differences must, however, be interpreted with caution since the variations in work load and thus oxygen uptake were wider for the patients than for the control subjects because of the varying symptomatology observed among the patients during the study. I the analyses of the exercise data the Fick equation solved for oxygen uptake was therefore entered as model and the variables of this equation were analyzed separately. The equation reads $\dot{V}_{O_2} = \dot{Q} \times$ (O_2 diff) where $\dot{Q} = F \times SV$ (F = heart rate, SV = stroke volume). I order to also elucidate the transition from rest to exercise and to magnify the range of the abscissa (V_{O_2}) the resting data were also included in the analysis.

The different variables in the Fick equation are subject to changes depending on the normal aging process (Granath et al. 1964, A). The patients have therefore been divided into two groups for the analysis and illustration of some of the circulatory data: patients <50 years of age, and patients >50 years of age.

The *relationship between cardiac output and oxygen uptake* during exercise (Fig. 7) varied among the *patients* from normokinetic to hypokinetic when compared with the control material. Eight patients developing angina pectoris were unable to increase their oxygen uptakes to levels comparable to the majority of the other patients or the control subjects (>750 ml/min.). These patients were therefore not included in the calculation of the regression lines for the cardiac output vs. oxygen uptake relation used for comparison with the control group or other studied groups which had wider ranging oxygen uptakes. The cardiac output during exercise in four of the *control subjects* was found to exceed 16 l/min. Two of these cases (no. 39 and 40) had normal oxygen uptakes in relation to the work load but low a-$\bar{v}O_2$ differences, that could not be explained by any apparent technical errors in the analysis of the blood samples.

The regression lines for the relationship between cardiac output and oxygen uptake for the patients and the control subjects are demonstrated in figure 8. There was no significant difference either in level or slope between the regression lines for the patients. When these regression lines were compared with the line for the control subjects differences could be demonstrated between the levels of the lines but not the slopes. The average difference in the level of the lines was 1.5 l/min. ($P<0.01$) between control subjects and patients below 50 years of age, and 2.0 l/min. ($P<0.05$) between control subjects and patients above 50 years of age.

tients with exercise tolerances ≥ 600 kpm/min only two (cases no 3 and 20) had sedentary occupations (Group I) the remaining eight had moderately strenuous or strenuous occupations (Group II—III) with probably higher requirements of physical activity in ordinary daily life Seven of these patients were thus functionally just as incapacitated as the patients belonging to Group I, with much lower observed exercise tolerances The exercise tolerance was not correlated to the *duration of symptoms* if all patients regardless of clinical diagnosis were included. Ten out of twelve patients with or without previous infarction and a disease history of 5 years had, however markedly low exercise tolerances. It is reasonable to assume that these low values could be partly explained by a long period of relative physical inactivity due to the symptoms (effort angina) This factor should, therefore be taken into account when the exercise tolerance tests for patients with, for instance, coronary heart disease are evaluated and compared with values obtained from healthy subjects

B. Hemodynamic investigations

Findings at rest and during exercise

Central hemodynamic studies were performed by means of right heart catheterization in the thirty-eight patients and the eleven control subjects included in this study Due to complications accounted for earlier under Methods, only thirty-six of the patients are included in the presentation of the hemodynamic findings Primary data from the cardiac catheterization of patients and control subjects at rest and during exercise, as well as in thirty cases after digitalis or saline administration, are presented in tables 5 and 6 Means and errors of the means, as well as standard deviations of the data obtained are presented separately in tables 7 and 8 (see Appendix)

At rest the patients had a normal mean oxygen uptake ($\dot{V}_{O_2}$) of 260 (S D = ± 39) ml/min or +13 % of the predicted basal oxygen uptake, that was transported by a cardiac output ($\dot{Q}$) of 6 1 (S.D = ± 1.2) l/min. with an extraction of oxygen per litre blood (*a* vO_2 *diff*) of 44 (S.D = ± 7) ml/l In the control group the corresponding data for V_{O_2} $\dot{Q}$ and a vO_2 diff were 268 (S.D = ± 30) ml/min. (+10 %) 7 3 (S.D = ± 2.1) l/min. and 38 (S.D = ± 8) ml/l. The mean stroke volume for the patients was 82 (S.D = ± 17) ml and for the control subjects 105 (S.D = ± 25) ml The difference between these two absolute values was significant ($P < 0\ 01$) The average heart rates for the patients and the control subjects were almost identical The average intracardiac, pulmonary and systemic pressures among the patients and the control subjects were essentially identical while both the average pulmonary and systemic vascular resistances were higher for the patients than for the control group ($P < 0\ 05$)

During exercise angina pectoris was precipitated in twenty-two of the thirty six patients during the initial exercise period and the studies were therefore discontinued In five cases the initial exercise work load could be doubled, and in four of those cases angina pectoris w:

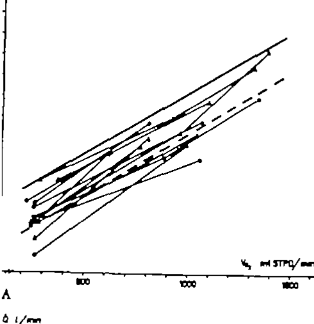
studies were discontinued after one work period and one study after two work periods because of general caution or marked intracardiac pressure elevations without chest pain. The arterial oxygen saturations during exercise ranged for the patients between 100 % to 91 %. One of the control subjects had an arterial oxygen saturation during exercise of 88 % that could not be explained by any methodological error and in spite of normal routine pulmonary function tests and a normal chest x-ray.

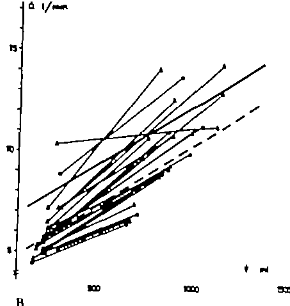
During exercise the patients had higher heart rates, lower stroke volumes, higher intraventricular pressures and systemic resistances than the control subjects. The mean differences for these variables in patients and control subjects were all highly significant ($P<0.001$). The mean patient cardiac output was also lower than that of the control subjects ($P<0.05$). The significance of these differences must, however be interpreted with caution since the variations in work load and thus oxygen uptake were wider for the patients than for the control subjects because of the varying symptomatology observed among the patients during the study. In the analysis of the exercise data the Fick equation solved for oxygen uptake was therefore entered as model and the variables of this equation were analyzed separately. The equation reads $\dot{V}_{O_2} = \dot{Q} \times$ (a-vO_2 diff) where $\dot{Q} = F \times SV$ (F = heart rate, SV = stroke volume). In order to also elucidate the transition from rest to exercise and to magnify the range of the abscissa ($\dot{V}_{O_2}$) the resting data were also included in the analysis.

The different variables in the Fick equation are subject to changes depending on the normal aging process (Granath et al. 1964, A). The patients have therefore been divided into two groups for the analysis and illustration of some of the circulatory data: patients <50 years of age, and patients >50 years of age.

The *relationship between cardiac output and oxygen uptake* during exercise (Fig. 7) varied among the *patients* from normokinetic to hypokinetic when compared with the control material. Eight patients developing angina pectoris were unable to increase their oxygen uptakes to levels comparable to the majority of the other patients or the control subjects (>750 ml/min.). These patients were therefore not included in the calculation of the regression lines for the cardiac output vs. oxygen uptake relation used for comparison with the control group or other studied groups which had wider ranging oxygen uptakes. The cardiac output during exercise in four of the *control subjects* was found to exceed 16 l/min. Two of these cases (no. 39 and 40) had normal oxygen uptakes in relation to the work load but low a-vO_2 differences, that could not be explained by any apparent technical errors in the analysis of the blood samples.

The regression lines for the relationship between cardiac output and oxygen uptake for the patients and the control subjects are demonstrated in figure 8. There was no significant difference either in level or slope between the regression lines for the patients. When these regression lines were compared with the line for the control subjects differences could be demonstrated between the levels of the lines but not the slopes. The average difference in the level of the lines was 1.3 l/min. ($P<0.01$) between control subjects and patients below 50 years of age and 1.0 l/min. ($P<0.05$) between control subjects and patients above 50 years of age.





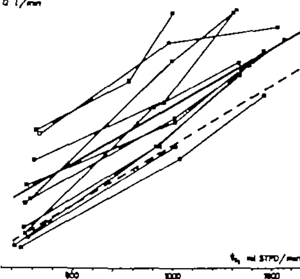


Fig. 7. Cardiac output (Q) in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest (open symbols) and during exercise (filled symbols) in supine position for 36 patients and 11 control subjects. A) patients <50 years of age. B) patients >50 years of age. C) control subjects. Regression lines represent the relation for young men (heavy line) (*Holmgren, personal communication*) and old men (broken line) (Granath et al. 1964 A). *Circles denote patients with* myocardial infarction, triangles patients with angina pectoris only and squares control subjects.

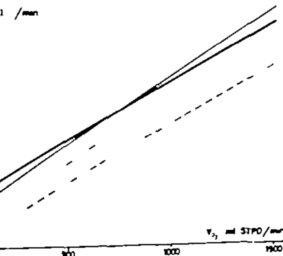
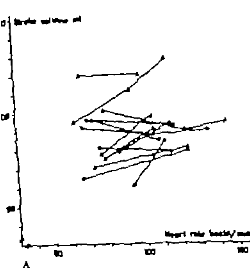
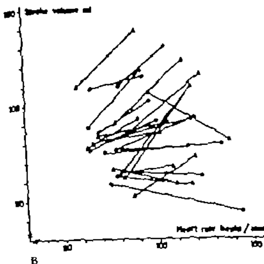


Fig. 8. Regression lines for cardiac output ($\dot{Q}$) on oxygen uptake ($\dot{V}_{O_2}$) for patients <50 years of age (dotted line), patients >50 years of age (broken dotted line) and control subjects (thin line). Heavy and broken lines represent the regression equations for young and old men as in Fig. 7. *Regression equations:* patients <50 years. $\dot{Q}=4.65+0.006\ \dot{V}_{O_2}$ (r=0.91, S.D.=±0.97, = 5). Patients >50 years $Q=4.57+0.007\ \dot{V}_{O_2}$ (r=0.86, S.D.=±1.48, n=34). Control subjects. $Q=5.54+0.007\ \dot{V}_{O_2}$ (r=0.84, S.D.=± .01, = 32).





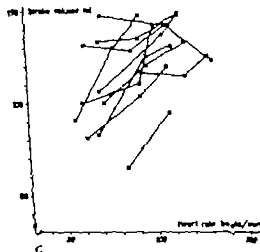
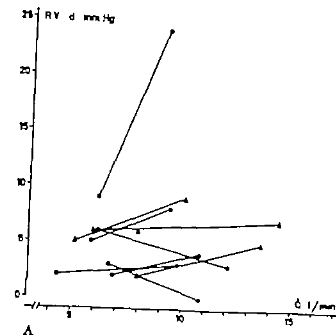
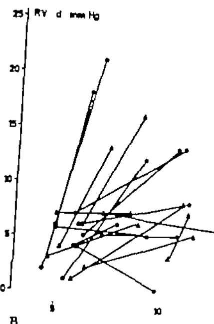


Fig 9 Stroke volume in relation to heart rate at rest (open symbols) and during exercise (filled symbols) for A) patients <50 years of age B) patients >50 years of age, C) control subjects. Symbols as in fig. 7

The a-O_2 diff increased in all cases during exercise The mean O_2 diff for the patients was 88 (S.D = ±14) ml/l and for the control subjects 81 (S.D = ±16) ml/l during light exercise (EI) and 89 (S.D = ±15) ml/l during moderate exercise (EII) The relation between the a-vO_2 diff and the oxygen uptake (V_{O_2}) is dependant on the cardiac output ($\dot{Q}$) as the Fick equation solved for a-O_2 diff reads a-O_2 diff $= \frac{V_{O_2}}{\dot{Q}}$ Differences in the relation between the a-vO_2 diff and $\dot{V}_{O_2}$ are thus dependant on the relations between the variables presented previously the high a-vO_2 differences observed among the patients being consequences of the hypokinetic circulatory conditions during exercise.

The *heart rate* during exercise were higher among the patients than among the





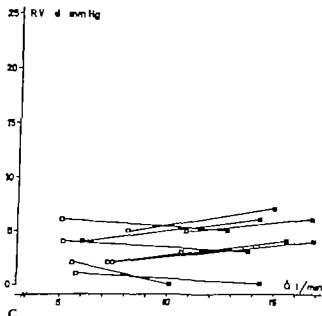


Fig 10 Right ventricular end-d pressure (RV_{ed}) in relation to card put (Q) at rest (open symbols) an ing exercise (filled symbols) for A tients <50 years of age B) patient years of age C) control subjects Sy as fig 7

control subjects when related to work load or oxygen uptake. In presence of a difference in the level of cardiac output vs. oxygen uptake between patients and the control subjects either due to lower heart rate (F) or stroke volume (SV) this difference in heart rates indicate that the low cardiac outputs among the patients were primarily due to small stroke volumes.

Light to moderate exercise produced increase in *stroke volume* in the majorit the patients and in the control subject seen in figure 9 The mean stroke vol during exercise for the patients was 92 (S $= \pm 22$) ml and 11 % higher than mean stroke volume at rest. The cor sponding figures for the control subje were 1 1 (S D $= \pm 15$) ml and 16 % T

of the patients had lower stroke volumes during exercise than at rest. This was also observed in one of the control subjects. In one of the patients (case no 15) and the control subject the fall was the result of a hyperkinetic resting circulation turning normokinetic during exercise

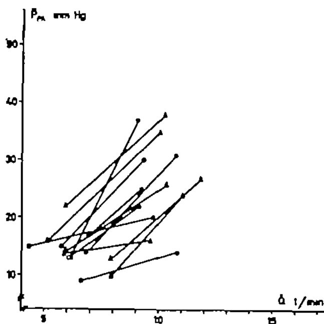
The transition from rest to exercise brought about an increase in the *right ventricular systolic pressures* in all subjects. The mean systolic pressure during exercise for the patients was significantly higher than the value for the control subjects ($P<0.001$) although the latter was obtained at a substantially higher blood flow (Exercise II)

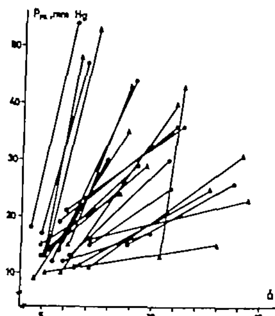
The *right ventricular end-diastolic pressures* for the patients, were in a few cases even at rest higher than what was observed in the control group. There was in the majority of cases an increase of the end-diastolic pressure in the right ventricle during exercise and the mean right ventricular end diastolic pressure for the patients was significantly higher ($P<0.01$) than the mean pressure for the control subjects, measured during the second exercise period. Ten patients had pressures that exceeded the values for the control subjects. As illustrated in figure 10 there was no difference in the pressure response between patients with previous myocardial infarctions versus those with no previous clinically recognized infarctions. The abnormal pressures (>9 mm Hg) were always associated with marked elevations of the pulmonary artery or pulmonary capillary mean pressures and thus no isolated right ventricular end-diastolic pressure abnormalities were observed.

During exercise the *pulmonary artery systolic pressures* measured simultaneously or directly after the pulmonary capillary venous pressures, were usually somewhat lower than the right ventricular systolic pressures measured at the end of the exercise period. The maximal systolic pressure gradient between the right ventricle and the pulmonary artery observed during exercise was 15 mm Hg. In six cases the systolic pressures in the pulmonary artery and the right ventricle late in the exercise period were somewhat lower than the systolic pulmonary artery pressures recorded simultaneously with the pulmonary capillary venous pressures. The *pulmonary artery mean pressures* rose in nineteen cases to levels exceeding the highest pressures observed in the control group both during light (EI) and moderate (EII) exercise as illustrated by figure 11. The mean value for the group of patients was significantly higher than both exercise mean values (EI or II) for the control group ($P<0.001$)

The PCV pressure could be recorded during exercise in thirty-two of the thirty-six patients and in ten of the eleven control subjects. In the remaining five cases the position of the catheter tip was such, that the curves were damped or similar to the pulmonary artery tracings obtained from the side hole. The PCV mean pressures exceeded the diastolic pressures in the pulmonary artery in four cases during exercise (no. 11 14 27 31) The maximal difference observed was 4 mm Hg. All four cases had prominent and dominating v waves not coinciding with the systolic peaks of the pulmonary artery pressure curves. In three of these cases the pressures in the pulmonary artery were markedly elevated the fourth had normal pressures but a PCV mean pressure of 20 mm Hg (case no 27)

The mean value for the PCV mean pressures during exercise for the patients was significantly higher than both the exercise





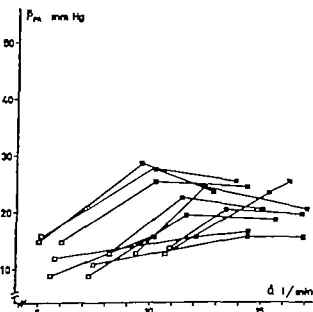
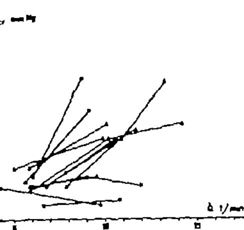
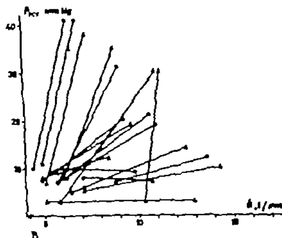


Fig. 11. The pulmonary artery mean pressure ($\bar{P}_{PA}$) in relation to cardiac output ($\dot{Q}$) at rest (open symbols) and during exercise (filled symbols). A) patients <50 years of age. B) patients >50 years of age. C) control subjects. Symbols as in fig. 7.

mean values for the control subjects ($P < 0.001$) (Fig. 12). The PCV pressures observed in the present material may be regarded as reflecting the pressure changes in the left atrium (Werkö and Lagerlöf 1950). Thus the PCV mean pressures observed would indicate that the pressures in the left atrium were elevated above normal levels in thirteen cases. Since these pressure elevations could not be a result of mitral valvular disease, they are in turn thought to indirectly reflect changes of the filling pressure in the left ventricle. The relation between the right ventricular end-diastolic





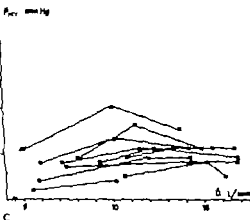


Fig. 12. The PCV mean pressure ($\bar{P}_{PCV}$) in relation to cardiac output ($\dot{Q}$) at rest and during exercise. A) patients <50 years of age B) patients >50 years of age, C) control subjects.

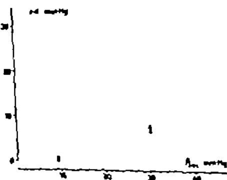
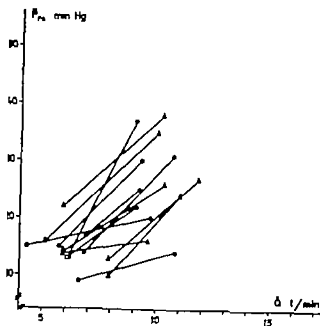
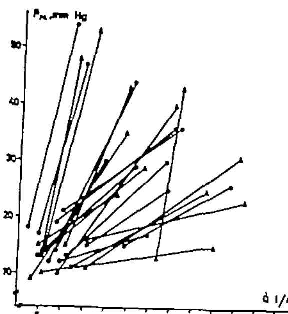


Fig. 13. The right ventricular end-diastolic pressure (RV_{ed}) in relation to PCV mean pressure ($\bar{P}_{PCV}$) during exercise. Symbols as in fig. 3.





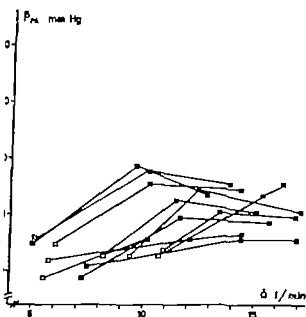


Fig 11 The pulmonary artery mean pressure ($\bar{P}_{PA}$) in relation to cardiac out put (Q) at rest (open symbols) and during exercise (filled symbols) A) patients <50 years of age, B) patients >50 years of age C) control subjects. Symbols as in fig 7

mean values for the control subjects (P <0 001) (Fig 12) The PCV pressures observed in the present material may be regarded as reflecting the pressure changes in the left atrium (Werkö and Lagerlöf 1950) Thus the PCV mean pressures observed would indicate that the pressures in the left atrium were elevated above normal levels in thirteen cases. Since these pressure elevations could not be a result of mitral valvular disease, they are in turn thought to indirectly reflect changes of the filling pressure in the left ventricle The relation between the right ventricular end-diastolic

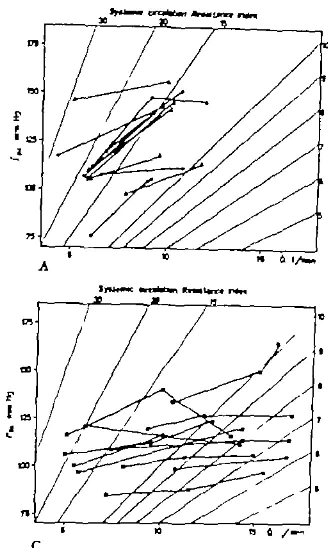
individual pressure changes are illustrated in fig. 14. The resistance response to exercise among patients and control subjects is also illustrated in this figure where the two parameters used for calculation of *the systemic resistance* are plotted against each other together with the isoresistance index lines. In all but one case the systemic resistances during exercise were lower than at rest. The systemic resistances observed during exercise were higher among the patients (M = 1090 dyn.sec.cm^{-5} S.D = ± 270) than among the control subjects during light or moderate exercise (EI M = 820 dyn.sec. cm^{-5} S.D = ± 170) (EII M = 630 dyn. sec.cm^{-5} S.D = ± 110). This could be result of the hypokinetic conditions, but also a result of differences in systemic vascular rigidity between patients and control subjects, the mean systemic pressures during exercise in the majority of patients being higher than in the control group at comparable levels of cardiac output.

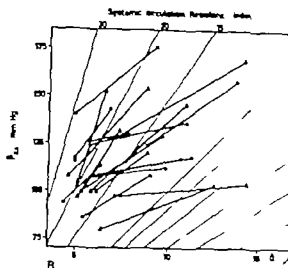
Comments. The central circulatory conditions observed among the patients differed in several respects from the conditions observed in the control group. These differences were most apparent during exercise. The differences could theoretically be due to biased selection of the control group including subjects with larger cardiovascular capacities than ordinary untrained subjects. In previous chapters it has, however been demonstrated that the present control material did not differ significantly from normally untrained subjects in the same age group regarding THb, heart volume or physical working capacity. It should, further more, be pointed out that the hemodynamic conditions in the control material both at rest and during light to moderate exercise were comparable to those observed for normal healthy subjects in earlier reports given by among others Hickam and Cargill (1948) Riley et al. (1948) Lagerlöf and Werkö (1948) Dexter et al (1951) Donald et al. (1955) and Holmgren et al. (1960 B)

Oxygen was *at rest* transported by a lower cardiac output in patients than in the control subjects (P>0.05) indicating that the circulatory conditions in patients with coronary heart disease were hypokinetic as compared to those of the control subjects. Similar findings have recently also been reported in studies of the circulation in healthy old men between 61 and 83 years of age by Granath et al. (1964, A) Low *stroke volume* were also observed among these old men and have previously also been found among patients with coronary heart disease most recently by Malmcrona et al. (1963) in follow up studies on patients with healed myocardial infarctions.

When the relationship between cardiac output and oxygen uptake *during exercise* among the studied subjects was compared with previous studies of similar type (Fig. 8) there was no significant difference between the regression line for the control subjects (mean age 49.5 years) and that of young men (Holmgren 1963) (personal communication) or between the regression lines for the patients and that of old healthy males studied by Granath et al. (1964, A)

The *stroke volume* is related to the dimension of the circulatory system in healthy subjects (Holmgren et al 1960, B). When the stroke volumes during exercise were compared with dimensional factors of the circulatory system, such as THb and heart volume the deviations from the regression lines and the S.D.s for these relations in young healthy subjects were more pronounced for the patients than for the





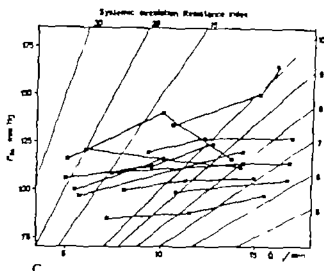


Fig 14 Brachial artery mean press re (P_{BA}) in relation to cardiac output (Q) at rest and during exercise. A) patients <50 years of age, B) patients >50 years of age, C) control subjects Symbols as in fig. 7 The oblique lines re isoresistance index lines.

pressures and the PCV pressures (Fig. 13) indicated that such changes ($\bar{P}_{PCV} > 20$ *mm* Hg) were present only in the left ventricle in four out of twelve cases, whereas in eight cases they were combined with end-diastolic pressure elevations in the right ventricle (>9 mm Hg)

As seen from the figures illustrating the *intracardiac pressure response during exercise* the most marked pressure elevations were found among the older patients However no correlation was found between intracardiac pressures and age for the present material

The pulmonary vascular resistances (PVR) for patients as well as control subjects were within the normal range (Lagerlöf Werkö 1948) The mean PVR for the patients was 90 (S D = ± 40) dyn sec cm^{-5} and for the control subjects 70 (S.D = ± 20) dyn sec cm^{-5} during light exercise (EI) and 60 (S.D = ± 20) dyn.sec cm^{-5} during moderate *exercise* (EII) There was no s gn fi ant difference between the two groups

The ***mean brachial artery mean p e during exercise*** was higher for the patients than for the control subjects The difference was however *not* significant (P>0 05) The

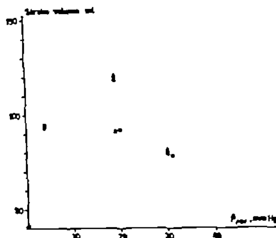


Fig 16. Stroke volume during exercise in relation to PCV mean pressure ($\bar{P}_{PCV}$) Symbols as in fig 7

control subjects in whom the total heart volume in the recumbent position was estimated, twenty out of twenty-nine patients, but only two out of eleven control subjects had stroke volumes that fell outside the 95% confidence interval for healthy subjects as demonstrated in figure 15 Thus a marked difference between stroke volume dimensions for patients and control subjects was present even when the influence of variations in total heart volumes was eliminated.

Decreasing stroke volumes upon the transition from rest to exercise in patients with coronary heart disease have previously been demonstrated by Malmcrona et al. (1963) Such a fall occurred both in patients who had, as well as in those who had not, returned their work after myocardial infarction and was regarded as sign of heart failure by the authors.

In order to study the relation between the absolute size of the stroke volume and the PCV pressure during exercise, all stroke volumes were compared with the corresponding PCV mean pressures (Fig. 16) All patients with higher PCV pressures than the control subjects had also smaller stroke volumes. A small stroke volume during exercise was however not necessarily combined with an abnormal PCV pressure, although the PCV pressures in the patients with stroke volumes<the controls were significantly higher ($P<0.01$) than in the patients with stroke volumes=the control subjects. It was thus evident from this comparison that small stroke volumes during exercise could exist without any apparent pressure abnormality among patients with small hearts but were in the majority of cases combined with elevated PCV pressures.

Elevated pressures in the pulmonary artery during light exercise in patients with coronary heart disease and normal sized hearts (<500 ml/m² BSA) have previously been observed by Müller and Rørvik (1958) They considered these pressure elevations to be a sign of acute left ventricular failure occurring in connection with angina pectoris and also observed a beneficial effect of nitroglycerin on both the pressures and the subjective symptoms. Intracardiac pressures during exercise of similar

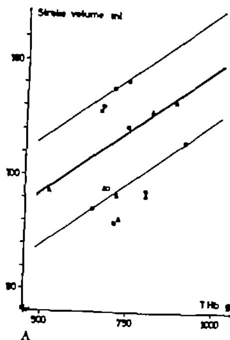
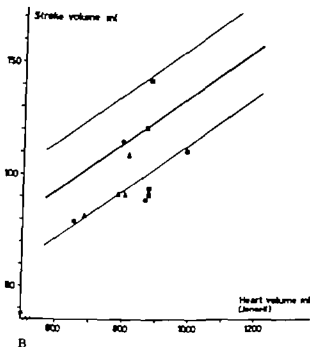
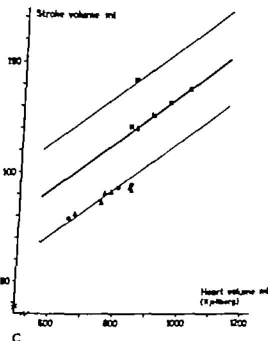


Fig. 15. Mean stroke volume during exercise in relation to A) total hemoglobin, B) observed heart volume, C) converted heart volume. Regression lines ±2 S.D. for healthy subjects (Holmgren et al. 1960, B). Symbols as in fig. 3.





control subjects as demonstrated in figure 15. Seventeen of thirty-six patients and ten of eleven control subjects had stroke volumes that fell within the 95 % confidence interval for the relation between stroke volume and THb observed for healthy subjects. The remaining nineteen patients had stroke volumes that fell below the 95 % confidence interval, and were thus low even when the variation in stroke volume due to differences in the total amount of hemoglobin was considered. Among the patients and the

however, not significant (P>0.01). The only significant difference observed among the rest values was in the right ventricular systolic pressures, which were lower during the second testing period than during the initial. This mean difference was, however, only 2.4 (S.D.$_d$ = ±1.69) mm Hg (P <0.01). During the second exercise period the mean oxygen uptake and the mean pulmonary artery mean pressure were lower than during the first. The mean difference for the oxygen uptakes was 56 (S.D.$_d$ = ±71) ml/min. and for the mean pulmonary artery mean pressures 0.8 (S.D. = ±0.83) mm Hg. These differences were, however, only probably significant (P<0.05). The remaining values were essentially unchanged and the mean differences lower than those at rest.

Comments. The present part of the circulatory studies was done in order to establish the degree of reproducibility of the hemodynamic findings both at rest and during exercise. The study was set up as to include the effect of previous exercise and one hour rest on the variables measured, so that the possible effect of any drug (in this study lanatoside C) could be more accurately evaluated. The variation coefficients calculated from the duplicate determinations include both the methodological and the biological variations taking place after exercise and during the recovery period.

The insignificant differences between the first and the second study at rest were not uniformly due to good reproducibility. Wide variations and thus large variation coefficients, in the majority of variables being >10 % occurred. During exercise these coefficients decreased to values <10 % in the majority of variables. These comparatively small errors indicated that the reproducibility of the findings was good due to a high degree of methodological and biological stability during the exercise study (Holmgren and Pernow 1960 A).

The effect of repeated exercise on the lesser circulation has earlier been studied by Widimsky et al. (1963) and by Granath et al. (1964, A). The first group of investigators studied four healthy volunteers and four patients with lung disease and only found significant changes in the pulmonary artery mean pressure ($\bar{P}_{PA}$) being lower both at rest and during exercise 30 to 45 minutes after the initial exercise period than before (P <0.005). The authors stated that their findings could be explained by the opening up of previously closed channels of the pulmonary vascular bed, induced by the increased blood flow and the slight increase in perfusion pressure remaining for an indeterminate period of time. Granath et al. studied the effect of previous exercise in the sitting position on the circulation at rest in the recumbent position approximately one hour later. In nine healthy old men they found a slightly lower stroke volume (P <0.05) and somewhat lower end-diastolic pressures in the right ventricle and lower pulmonary artery wedge pressures. The results of the present study show the same tendency although the wedge pressures were somewhat higher instead of lower.

magnitude as observed among the patients have also been reported to occur in old healthy men (Granath et al. 1964, A) These subjects had a hypokinetic circulation both at rest and during exercise but in contrast to the patients included in the present study the subjects with the highest pressures had the most marked increase of cardiac output in relation to their oxygen uptake The pressure elevations were thought to be results of an altered compliance of the ventricular walls perhaps due to a rigid collagenous connective tissue combined with high cardiac outputs due to high degrees of physical fitness In view of these differences the intracardiac pressure changes among the patients were not likely to be only a result of similar changes but could also be due to other changes in the myocardium occurring when blood supply during exercise became inadequate (see Discussion)

When *the systemic vascular resistances* of the patients were compared with those of hypertensive subjects studied by Varnauskas (1955) the present values were found to be within the range observed among hypertensives in functional Class I and II with normal hemodynamic findings in the lesser circulation but lower than those observed among the hypertensives belonging to functional Class III or IV who had abnormal pressures in the lesser circulation during exercise

The effect of previous exercise

In nine subjects the initial rest and exercise study was followed by one hour s rest whereupon the whole experimental procedure was repeated These patients were selected for this type of study prior to the initial rest and exercise study and regardless of the pressure response and symptomatology during the initial exercise period. The patients were given an intravenous injection of saline solution in the beginning of the rest hour as a placebo for the lanatoside C (Cedilanid®) given to twenty-one other patients.

Three of the patients had previous myocardial infarctions six had angina pectoris only The mean values for the anthropometric and clinical data for these nine patients were not significantly different from the mean values for the whole patient material or the twenty-one subjects who were given lanatoside C. Furthermore there was no significant difference between the hemodynamic findings either at rest or during exercise obtained in the initial study for this group and the lanatoside C patients

Mean differences, S.E. and S.D for the differences between the results of the first and second study are given in table 9 together with P values and the variation coefficients of single determinations (C) calculated from the duplicate determinations obtained with the present technique

The mean differences between the results obtained during the first and the second study were on the whole small both at rest and during exercise. *At rest* the cardiac outputs, the stroke volumes and the brachial artery mean pressures were lower during the second resting period than during the first The mean difference for the cardiac outputs was 0 73 ($S.D._d = \pm 1\ 022$) l min for the stroke volumes 13.2 ($S.D._d = \pm 13\ 08$) ml/beat and the $\bar{P}_{BA}$ s 7 1 ($S\ D._d = \pm 10\ 49$) mm Hg These differences were

ive symptoms was noted in nine (53 %) of the digitalized cases, who stated that they felt no, or in one case, definitely less pain during the second exercise period. No definite effect was produced by the saline injections. A positive effect upon the subjective symptoms was usually associated with significantly lowered pressures in the $\bar{P}_{PCV}$ ($\bar{d}=11$ mm Hg, $S.D._{\bar{d}}=\pm 3.8$ $n=8$, $P<0.001$) whereas in the cases in which no effect on the pain intensities was noted no significant lowering of the pressures was observed ($\bar{d}=3$ mm Hg, $S.D._{\bar{d}}=\pm 8.11$, $n=7$ $P>0.05$). Thus dif ference pressure response between the two groups was probably significant ($P<0.05$)

Comments The effect of acute digitalization on the hemodynamics at rest and during exercise in *healthy subjects or patient with non failing hearts* has previously been stud ied by many investigators. The most recent studies have been done by Williams et al. (1958) Selzer et al. (1959) Dresdale et al. (1959) Rodman et al. (1961) The effect on the cardiac output was studied by all authors No increase in cardiac output either at rest or during exercise could be noted. Instead, Williams found lowered cardiac out puts both at rest and during exercise one hour after digitalization, and Rodman transient drop in cardiac output at rest but no change in cardiac output during exercise. Lower resting heart rates after acute digita lization were observed by Williams, Selzer Rodman and during exercise only by Wil liams A small increase in average stroke olume at rest and during exercise was noted by Rodman.

The effect of digitalis on the intracardiac pressures at rest was studied by Dresdale and Selzer No significant effects on the right ventricular, pulmonary artery or PCV pressures either at rest or during exercise were noted.

In a recent survey of the hemodynamic effects of digitalis in the normal and diseased heart Rodman and Pastor (1963) concluded from their analysis of pertinent animal and human experiments that the action of digitalis was both extracardiac and car diac. The pharmacological activity was the same whether the heart was normal or diseased. It acted on the arteriolar smooth muscle and produced a rise in the systemic resistance and blood pressure. It also acted on the smooth muscle of veins causing a reduced venous return to the heart and therefore a drop in cardiac output (Ross et al. 1960) These effects were seen only after intravenous administration and were transient which could explain the above quoted findi gs. The action of digitalis on the myocardium produced increased force of systolic contraction (Braunwald et al 1961 B) In the non failing heart no increase in cardiac output either at rest or during exercise resulted from this direct myocardial effect as seen from the results of the quoted studies. It was thus concluded that acute ad ministration of a digitalis glucoside had no significant hemodynamic effects on indi viduals without cardiac disorders r on pa tients with non-failing hearts, that could be demonstrated with the present techniques.

It was of special nterest to the present study that these conclusions regarding the effect of digitalis also seemed valid for healthy old males with elevated intracardiac pressures, low cardiac outputs and stroke volumes during exercise, attributed to the normal aging process. This was demonstrated by Granath et al. (1964, A) who in thei studies noted no effect of acute digitalization

The effect of acute intravenous digitalization

Twenty-one of the thirty-eight subjects were given 1.2—1 6 mg of lanatoside C (Cedilanid®) intravenously a few minutes after the completion of the exercise period After a recovery period of one hour during which the subject rested on the catheteriza tion table the rest and exercise studies were repeated Mean differences, S.E., S.D and the P values for all differences between val ues obtained before and after digitaliza tion are given in table 10 (see Appendix)

Eleven of the subjects were patients with previous infarctions and ten had angina pectoris only No symptoms of acute in-toxication, induced by the drug administra tion, were noted in any of the tested subjects.

The results of the repeated studies done after acute digitalization were in contrast to the results obtained without digitaliza tion, significantly different from the initial results in several aspects. At *rest* the cardiac outputs were higher ($\bar{d}$=0 36 l/min. $S.D._d$ = ±0 72) and the right ventricular systolic pressures were lower ($\bar{d}$=2 3 mm Hg $S.D._d$ = ±4.43) after digitalis than before but the differences were not significant ($P < 0 05$) Significant differences in the re sults at rest were observed in the right ven tricular end-diastolic pressure, ($\bar{d}$=1 6 mm Hg, $S.D._d$ = ±2 06) ($P < 0 01$) the pul monary artery mean pressures ($\bar{d}$=2 6 mm Hg, $S.D._d$ = ±3.25) ($P < 0 01$) and the pulmonary capillary venous mean pressures ($\bar{d}$=3 0 mm Hg, $S.D._d$ = ±2 43) ($P < 0 001$) which all were lower after digitalis administration than before During *exercise* the cardiac outputs ($\bar{d}$=0 41 l/min., $S.D._d$ = ±0.81) and the right ventricular systolic pressures ($\bar{d}$=4 4 mm Hg $S.D._d$ = ±6.08) were lower but the differences were not significant ($P < 0 05$) The heart rates dur ing exercise after digitalis were significantly lower ($\bar{d}$=5.8 beats/min., $S.D._d$ = ±8 36) than before ($P < 0 01$) The right ventri cular end-diastolic (RV_{ed}) ($\bar{d}$=3.8 mm Hg, $S.D._d$ = ±3 62) the pulmonary artery mean ($\bar{P}_{PA}$) ($\bar{d}$=6.9 mm Hg, $S.D._d$ = ±7.92) and the pulmonary capillary venous mean ($\bar{P}_{PCV}$) ($\bar{d}$=6 7 mm Hg, $S.D._d$ = ±7 04) pressures were also significantly lower ($P < 0 001$)

The results indicate that even at rest acute digitalization had a small but signifi cant effect on both the flow and the intra cardiac pressures. During exercise the effect was more marked as evidenced by a highly significant lowering of the intracardiac pres-sures, and a significant lowering of the heart rate. No significant effects were de-tected on the oxygen uptakes, the a vO_2 dif ferences the stroke volumes, the systemic ar terial pressures or the vascular resistances. The individual pressure response of the drug varied widely In nine cases (43 %) there was no significant difference between the pressures whereas in twelve patients (57 %) there was a marked lowering of the intra cardiac pressures (>2 S.D of the mean difference between repeated exercise) There was no difference in the type of response be-tween patients with elevated intracardiac pressures and those with normal pressures, using the M+2 S.D for the control subject as limits.

Chest pain was recorded to have occurred during the initial exercise studies in seven-teen cases out of the twenty-one patients who were digitalized and in seven cases out of the nine patients who were given saline A positive effect of the drug upon the subject

ive symptoms was noted in nine (53 %) of the digitalized cases, who stated that they felt no, or in one case, definitely less pain during the second exercise period. N definite effect was produced by the saline injections. A positive effect upon the subjective symptoms was usually associated with significantly lowered pressures in the $\bar{P}_{PCV}$ ($\bar{d}=11$ mm Hg, $S.D._d = \pm 3.8$, $n=8$, $P < 0.001$) whereas in the cases in which no effect on the pain intensities was noted no significant lowering of the pressures was observed ($\bar{d} = 3$ mm Hg, $S.D. = \pm 8.11$ $n=7$ $P > 0.05$) This dif ference in pressure response between the two groups was probably significant ($P < 0.05$)

Comments The effect of acute digitaliza tion on the hemodynamics at rest and during exercise in *healthy subjects or patients with non failing heart* has previously been stud ied by many investigators. The most recent studies have been done by Williams et al. (1958) Selzer et al. (1959) Dresdale et al. (1959) Rodman et al. (1961) The effect on the cardiac output was studied by all authors. No increase in cardiac output either at rest or during exercise could be noted. Instead, Williams found lowered cardiac out puts both at rest and during exercise one hour after digitalization, and Rodman a transient drop in cardiac output at rest but no change in cardiac output during exercise. Lower resting heart rates after acut d gita lization were observed by Williams, Selzer, Rodman and during exercise only by Wil liams. A small increase in average stroke volume at rest and during exercise was noted by Rodman.

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It was of special interest to the present study that these conclusions regarding the effect of digitalis also seemed valid for healthy old males with elevated intracardiac pressures, low cardiac outputs and stroke volumes during exercise attributed to the normal aging process. This was demonstrated by Granath et al. (1964 A) who in their studies noted no effect of acut digitalization

Twenty-one of the thirty-eight subjects were given 1.2—1.6 mg of lanatoside C (Cedilanid®) intravenously a few minutes after the completion of the exercise period. After a recovery period of one hour during which the subject rested on the catheterization table the rest and exercise studies were repeated. Mean differences, S.E., S.D. and the P values for all differences between values obtained before and after digitalization are given in table 10 (see Appendix).

Eleven of the subjects were patients with previous infarctions and ten had angina pectoris only. No symptoms of acute intoxication, induced by the drug administration were noted in any of the tested subjects.

The results of the repeated studies done after acute digitalization were, *in contrast* to the results obtained without digitalization, significantly different from the initial results in several aspects. At *rest* the cardiac outputs were higher ($\bar{d}=0.36$ l/min., $S.D._d = \pm 0.72$) and the right ventricular systolic pressures were lower ($\bar{d}=2.3$ mm Hg, $S.D._d = \pm 4.43$) after digitalis than before but the differences were not significant ($P < 0.05$). *Significant differences in the results* at rest were observed in the right ventricular end-diastolic pressure, ($\bar{d}=1.6$ mm Hg, $S.D._d = \pm 2.06$) ($P < 0.01$), the pulmonary artery mean pressures ($\bar{d}=2.6$ mm Hg, $S.D._d = \pm 3.25$) ($P < 0.01$) and the pulmonary capillary venous mean pressures ($\bar{d}=3.0$ *mm Hg* $S.D._d = \pm$ -.43) ($P < 0.001$) which all were lower after digitalis administration than *before*. *During exercise* the cardiac outputs ($\bar{d}=0.41$ l/min., $S.D._d =$ ± 0.81) and the right ventricular systolic pressures ($\bar{d}=4.4$ mm Hg $S.D._d = \pm 6.08$) were lower but the differences were not significant ($P < 0.05$). The heart rates during exercise after digitalis were significantly lower ($\bar{d}=5.8$ beats/min., $S.D._d = \pm 8.36$) than before ($P < 0.01$). *The right ventricular* end-diastolic (RV_{ed}) ($\bar{d}=3.8$ mm Hg, $S.D._d = \pm 3.62$), the pulmonary artery mean ($\bar{P}_{PA}$) ($\bar{d}=6.9$ mm Hg, $S.D._d = \pm 7.92$) and the pulmonary capillary venous mean ($\bar{P}_{PCV}$) ($\bar{d}=6.7$ mm Hg, $S.D._d = \pm 7.04$) pressures were also significantly lower ($P < 0.001$).

The results indicate that even at rest *acute digitalization had a small but significant* effect on both the flow and the intracardiac pressures. During exercise the effect was more marked as evidenced by a highly significant lowering of the intracardiac pressures, and a significant lowering of the heart rate. No significant effects were detected on the oxygen uptakes, the a-vO_2 differences, the stroke volumes, the systemic arterial *pressures or the vascular resistances.* The individual pressure response of the drug varied widely. In nine cases (43 %) there was no significant difference between the pressures whereas in twelve patients (57 %) there was a marked lowering of the intracardiac pressures (>2 S.D. of the mean difference between repeated exercise). There was no difference in the type of response between *patients with elevated intracardiac* pressures and those with normal pressures, using the *M+2 S.D.* for the control *subject* as limits.

Chest pain was recorded to have occurred during the initial exercise studies in seventeen cases out of the twenty-one patients who were *digitalized* and in seven cases out of the nine patients who were given saline. A positive *effect of the drug* upon the subject

had, however, markedly low cardiac outputs, wide a O_2 differences and elevated intracardiac pressures, indicating the presence of myocardial failure already at rest in contrast to the present patients. As in the present study the response to the drug was not uniform, only 67 % of the patients were considered to have had a measurable effect of the drug. I twelve out of the present twenty-one cases (57 %) digitalis favorably affected the filling pressures of both the left and the right entricle without any signifi cant, concomitant changes of the stroke volumes as seen in fig 17 This may be taken as indirect evidence that the intracardiac pressure changes, noted during exercise before digitalization, could partly be due to acute myocardial failure occurring when the myocardium became overloaded during exercise (see Discussion) The small but significant effect of digitalization upon the circulatory dynamics at rest would furthermore indicate that a certain element of occult myocardial failure was present already at rest, in spite of the absence of any definite cardiac enlargement, roentgenological pulmonary congestion or peripheral edema.

C. Correlations between clinical, laboratory and hemodynamic findings

The present correlations between clinical and laboratory findings on one hand and the hemodynamic findings on the other were made in an attempt to analyze whether the routinely used clinical classifications and laboratory tests were correlated to the abnormal hemodynamic findings The hemodynamic ariables used for the correlations were the stroke volume, the right entricular end-diastolic pressure, and the PCV mean pressure during exercise These findings were related t the clinical diagnosis, the func tional lassification, the degree of previous physical activity and type of occupation, the ECG changes, the exercise tolerance and the oronary angiography

The present material consisted of eighteen patients with previous clinically recognized *myocardial infarcti* and eighteen patients with only *angina p ctori* When all individ ual data obtained during catheterization of the patients were analyzed for these two groups as seen in table 8 (see Appendix) ther was no significant difference between any of the mean values either at rest or during exercise. Furthermore, there was no difference in the response to acute digitalization between the two groups. It can therefore be concluded that in the present material there was no hemodynamic difference between the patients with and those without clinically recognized myocardial infarctions.

When the pertinent hemodynamic variables were analyzed in relation to *functional capacity* there was a difference in both mean stroke volume and mean filling pressures during exercise between the patients in Class I and II and those in Class III and IV The mean stroke volume duri g exercise was lower (P>0.05) and the pressures were higher (RV$_{ed}$ P<0.05) ($\bar{P}_{PCV}$ P<0.01) for the patients being severely incapacitated by angina pectoris (Class III—IV) when compared to those with only moderate

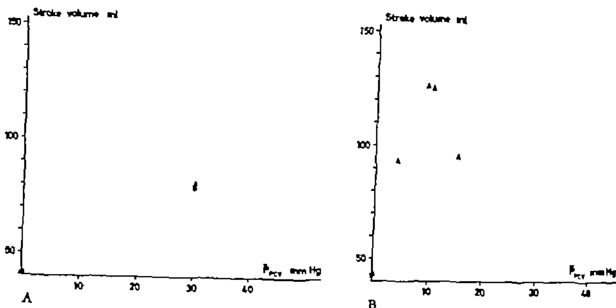


Fig 17 Stroke volume in relation to PCV mean pressure ($\bar{P}_{PCV}$) during exercise A) before digitalization, B) after digitalization. Circles denote patients with myocardial infarction, triangles patients with angina pectoris only

on the hemodynamics either at rest or during exercise in six elderly men.

The beneficial effects of acute digitalization on the hemodynamics at rest and during exercise in *patients with heart failure* are today well documented They consist of an increase in resting cardiac output, lowering of the heart rate increase of the stroke volume and lowering of the intracardiac and pulmonary artery pressures (Ferrer et al 1960 Lagerlöf and Werkö 1949 Selzer and Malmborg 1962)

The results of the present study indicated that digitalis had significant effects on the hemodynamics that were detected with the present technique This effect was different from what was observed in healthy subjects reported above. Instead it resembled the effect observed on the hemodynamics in patients with manifest or latent heart failure The effects of the drug upon the circulation at *rest* were thus *similar to those observed* in acute studies on patients with manifest heart failure resulting in increased cardiac outputs and lowered intracardiac pressures.

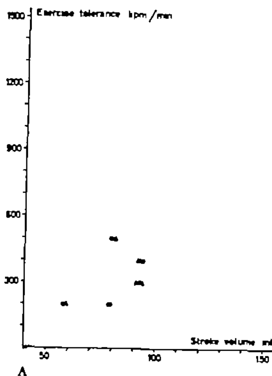
The hemodynamic effect on the circulation during *exercise* differed somewhat from the effect observed at rest since there was no average rise, but instead a small decrease in cardiac output secondary to the lower heart rate The *highly significant lowering* of elevated intracardiac and pulmonary pressures was not secondary to this lower cardiac output, since such pressure drops were not seen in the study of the reproducibility when similar decreases in cardiac output were observed. This effect of the drug can be compared with results of a study on the hemodynamics during exercise in latent cardiac failure (Selzer and Malmborg 1962) In that study acute digitalization caused a significant average increase of cardiac output, stroke volume lowering of heart rate and intracardiac pressures. These patients

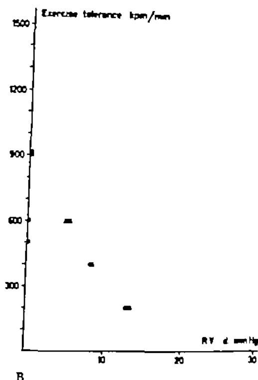
symptoms (Class I—II). There was no significant difference between the two groups of patients in the response to digitalis, as the lowering of the pressures during exercise was equal in both groups, and no significant change of the stroke volumes was noted in either group. These findings indicate that even among the patients with only moderate symptoms of coronary insufficiency (Class I—II) a certain element of myocardial failure, that in the majority of cases could be improved by digitalis, was present during exercise.

In an attempt to study the relation between *the previous physical activity* and the hemodynamic conditions observed among the patients, the values of these variables for the three groups were compared. The patients with low degree of previous physical activity (Group I) had an average stroke volume during exercise that was lower than in Group II or III. The difference between Group I and III was, however, only probably significant ($P < 0.05$). The pressures during exercise were higher in Group I than in II or III. The differences in right ventricular end-diastolic pressures were probably significant ($P < 0.05$) whereas the differences between the PCV pressures were not ($P > 0.05$). There were no differences in the response to digitalis between the three groups. The different degrees of previous physical activity seemed, therefore, not to have significantly influenced the incidence of myocardial failure during exercise.

Seventeen out of the thirty-six patients included in the hemodynamic study had abnormal mean QRS vector deflections in the electrocardiogram, whereas nineteen had normal QRS complexes. There was no significant hemodynamic difference during exercise between these two groups. When the patients were divided in one group with significant *ST depressions* during or 3 minutes after exercise and one group without ST depressions there was no significant difference between the hemodynamics during exercise. It can thus be stated, that patients with electrocardiographic signs of myocardial scarring or coronary insufficiency did not have lower stroke volumes or higher filling pressures during exercise than those without, in the present material of selected patients.

The individual *exercise tolerance* for the patients varied between 100 and 900 kpm/min, when estimated using the patients' own subjective symptoms as the limiting factor. When these values were compared with the exercise stroke volumes obtained during the catheterizations as seen in figure 18 it was seen that all ten patients with an exercise tolerance of ≥ 600 kpm/min. had stroke volumes that fell within the interindividual variations observed among the control subjects. The patients who had exercise tolerances < 600 kpm/min. had stroke volumes that were significantly lower ($P < 0.01$) than those observed in the control group. The right ventricular end-diastolic pressures for the patients with an exercise tolerance of ≥ 600 kpm/min. were all within the interindividual range observed in the control group and significantly lower ($P < 0.01$) than the pressure among the patients with exercise tolerances < 600 kpm/min. These abnormal pressures were observed only in cases with an exercise tolerance of ≤ 400 kpm/min. Nine patients out of ten with exercise tolerances ≥ 600 kpm/min. had PCV pressures within the range observed in the control group (≤ 20 mm Hg). The PCV pressures among twenty-two cases with an exercise tolerance < 600 kpm/





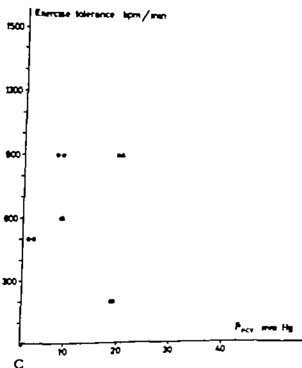


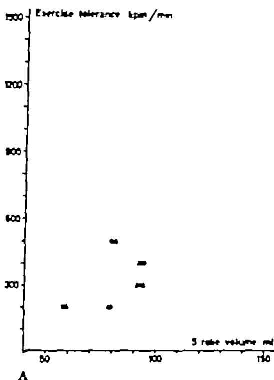
Fig. 18. Exercise tolerance in relation to A) mean stroke volume B) right ventricular end-diastolic pressure, C) PCV mean pressures ($\bar{P}_{PCV}$) during exercise for patients and control subjects. Symbols as in fig. 3

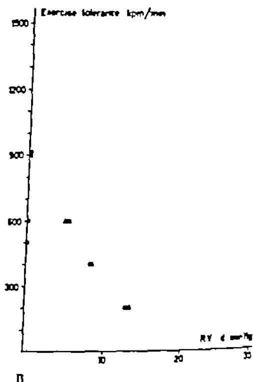
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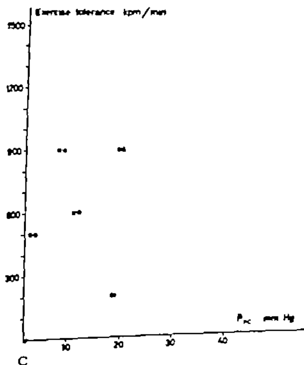


Fig. 18. Exercise tolerance in relation to A) mean stroke volume B) right ventricular end-diastolic pressure, C) PCV mean pressures ($\bar{P}_{PCV}$) during exercise for patients and control subjects. Symbols as in fig. 3.

patients with only one occlusion were analyzed according to location of the occlusion, there was no difference in either stroke volume or pressures between patients with occlusion of the right as compared with those with occlusion of the left coronary artery branches.

Comments. It was above demonstrated that there was no difference in hemodynamics between patients with clinically recognized myocardial infarctions or abnormal initial QRS vector deflections and those who had only angina pectoris or normal QRS complexes. The absence or presence of abnormal initial QRS vectors within the infarction group or angina pectoris group, furthermore did not significantly influence the hemodynamic variables within these groups. This lack of difference seemed therefore to indicate that in the present material healed myocardial infarctions per se clinically or electrocardiographically diagnosed did not significantly impair the cardiac function and that the functional changes were of similar degree for the groups. The most probable explanation for this is that the alternations in the myocardial function were primarily due to the atherosclerotic changes in the coronary vasculature which were of the same degree in the groups as demonstrated by coronary angiography.

The functional classification system was previously found to be valid for the differentiation of the hemodynamics in patients with hypertensive cardiovascular disease (Varnauskas 1955) and mitral stenosis (Eliasch 1952). Patients who belonged to Class III—IV had lower cardiac outputs and stroke volumes and higher intracardiac pressures than those belonging to Class I—II already at rest. There was also a marked [illegible] two groups indicating that the patients with the most pronounced subjective symptoms also had objective signs of a more advanced cardiac disorder. In the present material no such latter difference existed due to the selection of the patients. In spite of this there was a definite difference between the hemodynamics during exercise in functional Class I—II as compared to III—IV emphasizing the clinical usefulness of the functional classification system also among patients without any objective signs of myocardial failure other than the hemodynamic abnormalities during exercise.

The degree of previous physical activity was found to be positively correlated to the right ventricular end-diastolic pressures and the PCV pressures during exercise and positively influenced the increase in stroke volume during exercise among the old men studied by Granath and Strandell (1964, B). The findings in the present study were different from these correlations in that the intracardiac pressures were highest in the group with the lowest degree of previous physical activity instead of vice versa. However the degree of previous physical activity had no significant influence upon the hemodynamic conditions during exercise in the present material. This lack of difference between patients with a low and high degree of physical activity could probably be explained by the variations in the severity of the disease and the duration of the period with limited physical activity induced by the symptoms.

Granath and Strandell (1964, B) also correlated the degree of abnormality of ST segments to different hemodynamic variables and found only that old men with normal ST-segments during exercise had lower stroke volumes than those with ab-

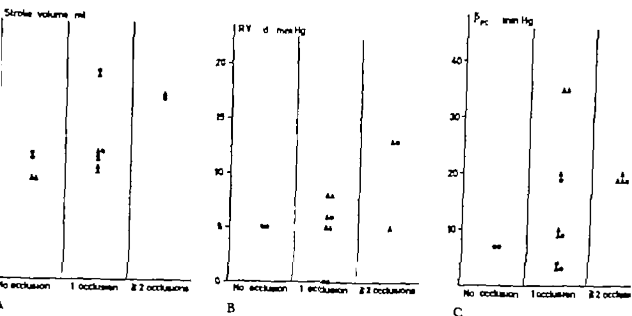


Fig 19 A) stroke olume, B) right entricular end-diastolic pressure (RV_{ed}) C) PCV mean pressure ($\bar{P}_{PCV}$) during exercise in relation to coronary angiographic changes grouped after number of occluded arteries (see Chapter V) Symbols as in fig 5

min. were in ten cases within the inter individual variations for the control subjects, whereas in twelve cases they were higher The difference in PCV pressures between the patients with exercise tolerances ≥ 600 kpm min. and those with < 600 kpm/min. was probably significant ($P < 0\ 05$)

Thus in can be stated that in the ten patients with relatively normal exercise toler ances (600—900 kpm/min) no definite hemodynamic abnormalities were detected during light to moderate exercise, with the exception of one case (no 12) who had an elevated PCV pressure during the second exercise period (E II) In contrast to this more than 50 % of the patients with low exercise tolerances (< 600 kpm/min.) had hemodynamic findings indicative of a hypokinetic circulation or myocardial failure during exercise

In thirty-one cases where *coronary angiographies* were done in connection with the other studies the angiographic findings were compared with the hemodynamic data. Using the grading system introduced in chapter V the patients were grouped after the number of arteries with Grade III changes (occlusions) In the present material the incidence of atherosclerosis in the coronary arteries observed with angiography was so high that any significant differences between patients with and those without occlusions could not be deducted only five out of thirty-one patients had angiograms in which there were no sign of occlusion. As seen in figure 19 there was furthermore no significant difference between the hemodynamic findings during exercise for patients with only one occluded artery as compared to those with two or all three (one case) main coronary arteries occluded although the pressure abnormalities were usually more marked among those with several occlusions. When the data for the

patients with only one occlusion were analyzed according to location of the occlusion, there was no difference in either stroke volume or pressures between patients with occlusion of the right as compared with those with occlusion of the left coronary artery branches.

Comments It was above demonstrated that there was no difference in hemodynamics between patients with *clinically recognized* myocardial infarctions or abnormal initial QRS vector deflections and those who had only angina pectoris or normal QRS complexes. The absence or presence of abnormal initial QRS vectors within the infarction group or angina pectoris group furthermore did not significantly influence the hemodynamic variables within these groups. This lack of difference seemed therefore to indicate that in the present material healed myocardial infarctions per se clinically or electrocardiographically diagnosed did not significantly impair the cardiac function and that the functional changes were of similar degree for the groups. The most probable explanation for this is that the alternations in the myocardial function were primarily due to the atherosclerotic changes in the coronary vasculature which were of the same degree in the groups as demonstrated by coronary angiography.

The functional classification system was *previously found to be valid for the differentiation* of the hemodynamics in patients with hypertensive cardiovascular disease (Varnauskas 1955) and mitral stenosis (Eliasch 1952). Patients who belonged to Class III—IV had lower cardiac outputs and stroke volumes and higher intracardiac pressures than those belonging to Class I—II already at rest. There was also a marked difference in heart size between the two groups indicating that the patients with the most pronounced subjective symptoms also had objective signs of a more advanced cardiac disorder. In the present material no such latter difference existed due to the selection of the patients. In spite of this there was a definite difference between the hemodynamics during exercise in functional Class I—II as compared to III—IV emphasizing the clinical usefulness of the functional classification system also among patients without any objective signs of myocardial failure other than the hemodynamic abnormalities during exercise.

The degree of previous physical activity was found to be positively correlated to the right ventricular end-diastolic pressures and the PCV pressures during exercise and positively influenced the increase in stroke volume during exercise among the old men studied by Granath and Strandell (1964, B). The findings in the present study were different from these correlations in that the intracardiac pressures were highest in the group with the lowest degree of previous physical activity instead of vice versa. However the degree of previous physical activity had no significant influence upon the hemodynamic conditions during exercise in the present material. This lack of difference between patients with a low and high degree of physical activity could probably be explained by the variations in the severity of the disease and the duration of the period with limited physical activity induced by the symptoms.

Granath and Strandell (1964, B) also correlated the degree of abnormality of ST segments to different hemodynamic variables and found only that old men with normal ST-segments during exercise had lower stroke volumes than those with ab-

normal ST segments. In the present study no significant differences were noted between patients with unchanged ST-segments during or 3 minutes after exercise, and those with significant ST depressions in connection with exercise. This lack of difference was again most likely a result of the selection of the material, the majority of patients having either initial QRS vector or ST-T segment vector abnormalities already in the ECG at rest, obscuring the electrocardiographic reaction during exercise.

The exercise tolerance (work load at maximal performance) was not found to be significantly correlated to any of the circulatory parameters in Granath's and Strandell's study of the relationship between pressure and flow in old men. In contrast to this the result of the exercise tolerance test was found to be the most reliable indicator for the exclusion of abnormal circulatory conditions during exercise in the present group of patients. All ten patients (28 %) with an exercise tolerance $\geq$600 kpm/min had normal stroke volumes and intracardiac pressures during light exercise, whereas in the remaining twenty-six (72 %) with exercise tolerances $<$600 kpm/min. the hemodynamic findings during exercise were abnormal in at least fourteen cases (54 %). The present results are in general accordance with those reported by Malmcrona et al. (1963) comparing patients with unlimited working capacity and with limited working capacity after myocardial infarction to normal subjects. The exercise tolerance test seems, therefore, valuable in differentiating patients with coronary heart disease with normal circulatory conditions from those in whom myocardial failure could be suspected to occur during exercise.

Studies on the relation between findings at coronary angiography and hemodynamic parameters have previously been preliminary reported by Messer et al. (1961) and Ross et al. (1962). These reports were brief and were based upon a very limited number of case. Therefore, the reports cannot be used as a comparison with the present results. From observations in the present limited material of patients, it can be concluded that the differences seen in the coronary angiographies, analyzed crudely for only occlusions, seemingly did not influence the hemodynamics during exercise among the thirty-one patients studied. This could probably best be explained by the fact that the present grading system of the coronary angiography did not include the collateral circulation and did not reflect changes in the coronary blood flow during exercise. Consequently it could not be used as a quantitative measure of coronary insufficiency or myocardial hypoxia.

DISCUSSION OF RESULTS

All *Laboratory investigations* undertaken have conclusively shown that there were no significant differences between the patients with previously known myocardial infarctions and those without. The criteria for the selection of the patients excluded all patients with obvious cardiac enlargement or previous episodes of heart failure. Thus, the infarction group only contained patients who had relatively mild infarctions that had left no obvious signs of myocardial failure after the acute stage. It is furthermore well known that patients with coronary heart disease have clinically silent episodes of myocardial infarction (Stokes and Dawber 1959) This could have occurred in both groups in an unknown number of cases, but there was, as previously mentioned, electrocardiographic evidence suggestive of myocardial infarction in six of the nineteen patients in whom clinical diagnosis of myocardial infarction was not definitely established. The lack of difference between the two groups can, therefore, probably best be explained by the selection of the material and certain degree of clinical diagnostic inaccuracy including only patients with relatively small infarctions in one group and in the other those with previous silent infarctions and/or coronary insufficiency

The main purpose of *the hemodynamic studies* was to obtain hemodynamic data during coronary insufficiency brought about or accentuated by different degrees of physical exercise. The results showed that the subjective symptoms of coronary insufficiency (e.g., angina pectoris) during exercise were sometimes observed in patients with a hypokinetic circulation but with no marked intracardiac pressure elevations. On the other hand there were some patients in whom significant intracardiac pressure elevations occurred during exercise, without the presence of subjective symptoms of coronary insufficiency These findings indicated that angina pectoris was not caused by the elevated intracardiac pressures, nor necessarily a symptom of ventricular failure. Instead it can be assumed that the pain and the elevated pressures were caused by underlying myocardial changes and occurred independant of each other, though commonly occurring simultaneously during exercise.

The intracardiac pressure changes noted during exercise were probably dependant upon several different structural changes within the myocardium, altering the compliance of the ventricular walls and the functional properties of the ventricles. The pressure elevations could be explained by 1) changes in the connective tissue elasticity occurring during the normal aging process (Kohn et al. 1959) 2) myocardial hypertrophy (Harvey et al. 1962) 3) diffuse fibrosis in the myocardium known to occur in patients with coronary heart disease (Robin et al. 1956) 4) localized fibrosis in the scars after myocardial infarctions and 5) alterations in the distension of the myocardial fibres that occurred when the myo-

normal ST segments. In the present study no significant differences were noted be *tween patients with unchanged ST-segments* during or 3 minutes after exercise, and *those with significant ST depressions in* connection with exercise This lack of dif *ference was again most likely a result of* the selection of the material, the majority of patients having either initial QRS vector or ST T segment vector abnormalities already in the ECG at rest, obscuring the electrocardiographic reaction during exercise

The exercise tolerance (work load at maximal performance) was not found to be significantly correlated to any of the circulatory parameters in Granath s and Strandell s study of the relationship between pressure and flow in old men. In contrast to this the result of the exercise tolerance test was found to be the most reliable indicator for the exclusion of abnormal circulatory conditions during exercise in the present group of patients All ten patients (28 %) with an exercise *tolerance* $\geq$ *600 kpm/min.* had normal stroke volumes and intracardiac pressures during *light exercise, whereas in the re*maining twenty six (72 %) with exercise *tolerances* $<$ *600* kpm/min. the hemodynamic findings during exercise were abnormal in at least fourteen cases (54 %) The present results are in general accordance with those reported by Malmcrona et al. (1963) comparing patients with unlimited working capacity and with limited working capacity after myocardial infarction to normal subjects The exercise tolerance test seems, *therefore,* valuable *in differentiating* patients with coronary heart disease with normal circulatory conditions from those in whom myocardial failure could be suspected *to occur during exercise.*

Studies on the relation between findings *at coronary angiography and hemodynamic* parameters have previously been preliminary reported by Messer et al. (1961) and Ross et al (1962) These reports were brief and were based upon a very limited number of case Therefore, the reports cannot be used as a comparison with the present results. From observations in the present limited material of patients, it can be concluded that the differences seen in the coronary angiographies, analyzed crudely for only occlusions, seemingly did not influence the hemodynamics during exercise among the thirty-one patients studied *This could probably* best be explained by the fact that the *present grading system of the coronary* angiography did not include the collateral circulation and did not reflect changes in the coronary blood flow during exercise Consequently it could not be used as a quantitative measure of coronary insufficiency or myocardial hypoxia.

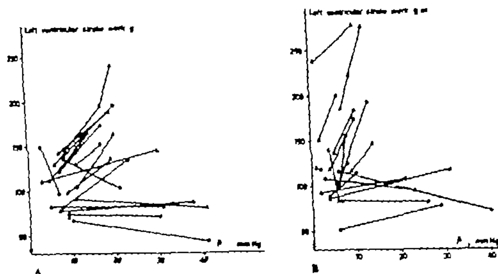
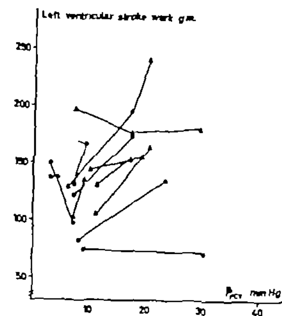
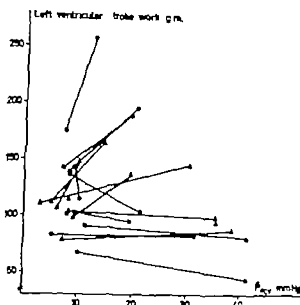


Fig. 21 Left ventricular stroke work in relation to PCV mean pressure ($\bar{P}_{PCV}$) at rest and during exercise A) before digitalisation, B) after digitalisation. Symbols as in fig. 7

increase in stroke work in all control subjects and the majority of the patients. In ten patients the stroke work was however unchanged or decreased when their cardiac outputs increased during exercise. All these cases had also elevated $\bar{P}_{PCV}$ pressures making the work pressure relation markedly abnormal. It could therefore be surmised that these pressures were not only a result of stiffness or hypertrophy of the ventricular walls (1—4) but also due to other myocardial factors resulting in an abnormal myocardial function (Berglund 1955). In a discussion of the response of the abnormal heart to exercise by Harvey et al. (1962) it was concluded that a normal flow response but abnormal pressure response in the lesser circulation on effort (a normokinetic—hyperpyeic circulation) could be ascribed to hypertrophy of the myocardium, while a response with both abnormal flow and pressures (hypokinetic—hyperpyeic circulation) such as observed in about one third of the present patients, was postulated to indicate altered myocardial function and ventricular dilatation.

The overall effect of acute digitalization on the hemodynamic parameters during exercise supported the assumption that a certain element of myocardial failure was present among the patients with abnormal work vs. filling pressure, since digitalis lowered the filling pressures at unchanged or increased levels of stroke work (Mc Michael 1944) (Figure 21). This could be result of better force of systolic ejection (improved contractility) inhibiting the postulated distension of the myocardial fibres and should then also be accompensed by an increase in the stroke volume of the left ventricle, the effective size of which could be within the methodological variations (C=





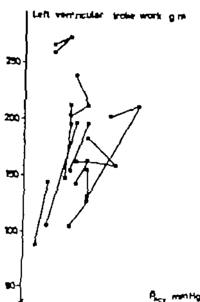


Fig. 20 Left ventricular stroke work in relation to PCV mean pressure ($\bar{P}_{PCV}$) at rest and during exercise for A) patients <50 years of age B) patients >50 years of age. C) control subjects. Symbols as in fig. 7

cardium became hypoxic (ventricular dilatation) The hemodynamic effects of such alterations might not be apparent when the external work of the heart is relatively low at rest, but only be detected when the output load is increased during exercise. This was demonstrated by a comparison between cardiac work and filling pressure at rest and during exercise in *figure 20* where the left ventricular stroke work expressed in *cgs* units

$$\frac{SV \times (\bar{P}_{RA} - \bar{P}_{PCV}) \times 13.6}{100}$$

was plotted against the P_{PC} reflecting the filling pressures in the left ventricle. As seen in this figure the transition from rest to exercise brought about an

and nitroglycerin both could alter the relation between oxygen supply and demand in the cardiac muscle so that the occurence of hypoxia and consequently pain could be prevented. The effectiveness of digitalis therapy with oral administration of a maintenance dose could be evaluated with double blind technique and follow up studies employing exercise tolerance tests, performed as in the present study

8 %) and, therefore, not detected with the present technique Even such a small increase of the stroke volume for the left ventricle would undoubtedly lead to a decrease in the *exercise end-diastolic* blood volume in the left ventricle and diminish the degree of the postulated ventricular dilatation. A fall in filling pressure and increase of stroke work was also observed in some of the cases with a normal work/pressure (function curve) relationship These findings indicated that improved myocardial contractility during increased cardiac work was achieved by digitalis administration also in patients with coronary heart disease without any definite hemodynamic signs of myocardial failure This is in contrast to several reports on the lack of effect of digitalis in such cases (Lagerlöf and Werkö 1949) (Harvey et al 1951) based primarily on data obtained at rest.

In five patients in the present material there were no effects of digitalis on the markedly increased intracardiac pressures or the low stroke volumes This lack of effect of the drug could have several different explanations Firstly it may be due to an insufficient amount of drug administered This explanation was not likely since the patients were given 1 2—1 6 mg of Cedilanid® which was more than what has previously been proven to give effect on patients with decompensated heart disease (Lagerlöf Werkö 1949) Secondly it may be explained by too short an interval between administration and study This explanation seemed unlikely since an inotropic effect of the drug has been observed within 30—60 minutes after the administration in patients with decompensated heart disease (Lagerlöf Werkö 1949) However full effect of an intravenous digitalization with lanatoside C is not obtained until after 2—3 hours according to Lown and Levine (1954) Thirdly the pressure elevations could be primarily due to altered compliance because of diffuse or localized fibrosis instead of overdistension of the myocardial fibres. This explanation seemed possible for the present material, but could not be *confirmed in any other way* Fourthly an intracellular electrolyte disturbance could have been present in these cases (Page 1964)

The positive effect of nitroglycerin on angina pectoris has been well documented in numerous reports. Muller and Rørvik (1958) and Johnson et al. (1959) have also demonstrated an inhibiting effect of nitroglycerin upon pulmonary pressure elevations coinciding with the anginal attacks, and thought to be due to left ventricular failure The precise mechanisms of the pain relieving and of the hemodynamic properties of nitroglycerin are controversial It is thought that among the beneficial effects are the lowering of peripheral resistance and the reduction of cardiac output, which decrease the cardiac work and the myocardial oxygen requirement (Gorlin et al 1959 Gorlin 1962 Marchetti et al 1964)

In 1934 the beneficial effect of a digitalis preparation on angina pectoris was reported by Edens. No more recent controlled studies of the effect of digitalis on angina pectoris in patients without congestive heart failure are, however known to the author It is known that digitalis improves the ability of the cardiac muscle to utilize available energy (Olson 1961) This leads to improved myocardial contractility and perhaps also to less intracellular hypoxia during increased cardiac work, which could explain the positive effect upon the subjective symptoms thought to be hypoxic in origin. Therefore, digitalis

failure. Among the remaining ten patients in whom angina pectoris was not recorded during exercise one had abnormal pressures whereas five had only low stroke volumes and four no hemodynamic abnormality

From these figures it was concluded that the limiting factor for the cardiac performance during exercise in the present study was isolated angina pectoris in 33 % angina pectoris and altered myocardial function in 39 % isolated altered myocardial function in 3 while 14 % only had a hypokinetic circulation, possibly limiting their performance Only 11 % were both subjectively and objectively free from any cardiac symptoms or signs during the hemodynamic study

A strong relation between altered myocardial function and angina pectoris was present 93 % of the cases with altered function having coincident angina pectoris and ice ersa in 54 % of the cases

A beneficial effect of digitalis upon the hemodynamics could be demonstrated in 57 and on the subjective symptoms in 53 f the studied cases. The lack of effect of the drug in the remaining cases could not be explained, but has been observed in approximately the same proportion in many similar studies as previously pointed out by Selzer and Malmborg (1962) The presented results prove that digitalis therapy was actually of value in the therapy of a certain number of patients with coronary heart diseases even when clinical signs of congestive heart failure were lacking.

The usefulness of both the functional classification system and the exercise tolerance test for evaluation of the hemodynamic conditions in patients with coronary insufficiency was demonstrated in the correlation studies. There were however wide hemodynamic variations within the different functional classes whereas no definite signs of myocardial failure during light exercise were evident unless the exercise tolerance was <600 kpm/min. This test should, therefore, be of greater clinical importance than the functional classification system for the selection of patients and therapeutic actions in a reconditioning program aimed at improving the circulatory function in cases of healed myocardial infarctions or coronary insufficiency (Biörck 1964) (WHO Report No 270 1964)

Analyses of the relation between the hemodynamics during exercise and the findings at coronary angiography showed that no conclusions egarding the cardiac performance could be drawn from the angiographic pattern in the present small and selected material employing the present grading system.

VIII

GENERAL CONCLUSIONS

The present study was undertaken in order to analyze some circulatory and hemodynamic findings in patients with coronary heart disease without clinical evidence of congestive heart failure and to relate these to the findings in a group of healthy subjects of the same age Furthermore the purpose was to relate the hemodynamic findings to pertinent clinical and laboratory findings in order to analyze which of these were the most important in the evaluation of cardiac function in this type of patients

In the analysis of the *general circulatory data* it could be demonstrated that the coronary heart disease apparently had no significant influence upon the total amount of hemoglobin as there was no significant difference in mean values for this variable between patients and control subjects It could, furthermore be concluded that within the present group of selected patients the underlying disease did not significantly influence the relation between total heart volume and amount of hemoglobin. Analysis of the ECG at rest during or 3 minutes after exercise revealed abnormalities indicative of coronary heart disease in thirty seven of the thirty-eight patients as compared to twenty three when only the ECG at rest was analyzed. It could, therefore, be stated that exercise and postexercise ECG were of definite importance for the electrocardiographic diagnosis of the cardiac disorder

The difference between mean exercise tolerance for the patients and the cont subjects was highly significant. No signi cant difference in exercise tolerance w noted between patients with and witho clinically recognized previous infarctio indicating that healed infarctions per did not influence this test among the studi subjects.

In the *hemodynamic studies* it was d monstrated that even at rest there was significant difference in stroke volumes be tween the patients and the control grou also resulting in a lower mean cardiac ou put and higher mean a vO difference In n cases were there any marked pressure eleva tions either in the right ventricle or in th pulmonary artery indicative of a markedly altered myocardial function during basal conditions During exercise the majority of the patients had hypokinetic circulations as evidenced by lower cardiac outputs in rela tion to oxygen uptake and lower stroke volumes than the control subjects This relationship was not influenced by age within the age range studied as there was no significant difference between patients 40—49 years of age and those 50—58 years of age

Angina pectoris was precipitated in twenty six of the thirty six subjects studied during exercise of varying intensity Twelve of these cases had normal left and right ventricular filling pressures during exercise, the remaining fourteen all had pressure elevations suggestive of acute ventricular

heart disease. Electrocardiographic changes during or 3 minutes after exercise indicating coronary insufficiency were present in 82 % of the cases with subjective symptoms thereof. *The exercise tolerance test* precipitated subjective symptoms of coronary insufficiency in thirty four (89 %) of the thirty eight studied patients, but in none of the control subjects. The average exercise tolerance for the patients was significantly lower than the average work load at maximal performance for the control subjects (P <0.001). No difference was noted between patients with and those without previous clinically recognized infarctions. Patients with slight to moderate limitation of the functional capacity (Class II) according to the N.Y.H.A. classification system had higher average exercise tolerance than those with marked limitation (Class III—IV) (P <0.001). No significant differences between the exercise tolerances for patients with different degrees of previous physical activity were noted. *All coronary angiographies* showed atherosclerotic changes in the coronary arteries. Twenty-six out of thirty-one patients had one or more occlusions of the three major coronary arteries. There was no apparent angiographic difference between patients with and those without clinically recognized infarctions.

B. The *hemodynamic investigation* demonstrated that the majority of the patients had hypokinetic circulatory conditions with low *cardiac output and stroke volume* both at rest and during exercise when compared with the control subjects. At rest there was no significant difference in the *intracardiac or extracardiac blood pressures* between the patients and the control subjects. Light to moderate exercise, precipitating angina pectoris in twenty-six out of thirty-six patients, elevated the ventricular filling pressures to levels above the range of the control subjects in at least fifteen cases (42 %). Abnormal filling pressures in the right ventricle ($\geq$9 mm Hg) were present in ten cases (28 %) and as indirectly measured using the PCV pressures in the left ventricle (>20 mm Hg) in thirteen cases (36 %). The abnormal pressures in the left ventricle were predominantly found in patients in whom no increases in stroke volume or left ventricular stroke work were observed upon the transition from rest to exercise. The pressure elevations were thus thought to be indicative of left ventricular failure. *The reproducibility* of the hemodynamic findings was good. There was no significant change in the hemodynamic effects of exercise in the same patient if studied in an identical fashion after an one hour recovery period. *Acute digitalization* had a significant effect upon the hemodynamics of the patients both at rest and during exercise. At rest the cardiac output was higher, the stroke volume was unchanged and the intracardiac pressures were lower after digitalization than before. During exercise, at identical work loads, the heart rate was lower, the stroke volume unchanged, the cardiac output consequently somewhat lower and the intracardiac pressures markedly lower (P <0.001). These results were thought to indicate improved myocardial function in twelve cases (57 %) whereas in nine cases (43 %) no improvement was noted. The occurrence of *angina pectoris* was prevented in nine (53 %) out of seventeen patients. This favorable effect of the drug was usually associated with improved myocardial function.

C. There was no significant hemodynamic difference between patients with and those

IX

SUMMARY

The main purpose of the investigation was to study a group of patients with coronary heart disease without clinical evidence of congestive heart failure. Thirty eight men with coronary heart disease and eleven male control subjects between 40 to *60 years of age* were included in the study.

Material

The *patient group* was selected to include only subjects with symptoms of coronary insufficiency *but with sinus rhythm* and without pulmonary dysfunction, valvular heart disease, significant hypertensive cardiovascular disease, left ventricular hypertrophy or cardiac enlargement. The *control subjects* were selected from volunteers and patients without significant cardiovascular disorders but within the same range of age *and physical activity as the patient group*.

Methods

The clinical investigation included a complete history with special attention paid to the occurrence of myocardial infarctions, the degree of previous physical activity, the functional capacity, the duration of symptoms *and a complete physical examination*. *The laboratory investigations* included in the study were: estimation of the total amount of hemoglobin, the relative and absolute heart volume, recording of multiple lead ECG at rest, during and after exercise and estimation of the exercise tolerance on a bicycle ergometer. In thirty-one of the patients *coronary angiographies* were performed. These were interpreted and graded by a roentgenologist (Gunnar Törnell, M.D.) *having no knowledge of the individual case* histories or other data concerning the patients.

The hemodynamic studies were done with the right heart and the peripheral systemic artery catheterization technique. Hemodynamic data were obtained at rest and during exercise, aimed at producing *angina pectoris in the patient group*. The control subjects were studied at rest and during light and moderate exercise. In nine patients the effect of previous exercise was investigated by a repeated study one hour after the initial study. Twenty-one patients were digitalized with 1.2—1.6 mg lanatoside C (Cedilanid®) after the initial study was *completed and then restudied one hour later*.

Results

A. The results of *the laboratory investigations* revealed that in ten patients and three control subjects *the total amount of hemoglobin* was lower than that predicted from the body surface area. Four of the patients and one control subject had *total heart volumes* measured in the recumbent position that were larger than those predicted from body weight, *total amount of hemoglobin or body* weight and age. In thirty-seven of the thirty eight studied patients, but in none of the control subjects, there were objective *electrocardiographic changes* indicative of coronary

heart disease. Electrocardiographic changes during or 3 minutes after exercise indicating coronary insufficiency were present in 82 % of the cases with subjective symptoms thereof. *The exercise tolerance test* precipitated subjective symptoms of coronary insufficiency in thirty four (89 %) of the thirty eight studied patients, but in none of the control subjects. The average exercise tolerance for the patients was significantly lower than the average work load at maximal performance for the control subjects (P <0.001). No difference was noted between patients with and those without previous clinically recognized infarctions. Patients with slight to moderate limitation of the functional capacity (Class II) according to the N.Y.H.A. classification system had a higher average exercise tolerance than those with marked limitation (Class III—IV) (P <0.001). No significant differences between the exercise tolerances for patients with different degrees of previous physical activity were noted. *All coronary angiographies* showed atherosclerotic changes in the coronary arteries. Twenty-six out of thirty-one patients had one or more occlusions of the three major coronary arteries. There was no apparent angiographic difference between patients with and those without clinically recognized infarctions.

B. The hemodynamic investigation demonstrated that the majority of the patients had hypokinetic circulatory conditions with low *cardiac output and stroke volume* both at rest and during exercise when compared with the control subjects. At rest there was no significant difference in the *intracardiac or intravascular blood pressure* between the patients and the control subjects. Light to moderate exercise, precipitating angina pectoris in twenty-six out of thirty-six patients, elevated the ventricular filling pressures to levels above the range of the control subjects in at least fifteen cases (42 %). Abnormal filling pressures in the right ventricle (≥9 mm Hg) were present in ten cases (28 %) and as indirectly measured using the PCV pressures in the left ventricle (>20 mm Hg) in thirteen cases (36 %). The abnormal pressures in the left ventricle were predominantly found in patients in whom no increases in stroke volume or left ventricular stroke work were observed upon the transition from rest to exercise. The pressure elevations were thus thought to be indicative of left ventricular failure. *The reproducibility* of the hemodynamic findings was good. There was no significant change in the hemodynamic effects of exercise in the same patient if studied in an identical fashion after an one hour recovery period. *Acute digitalization* had a significant effect upon the hemodynamics of the patients both at rest and during exercise. At rest the cardiac output was higher, the stroke volume was unchanged and the intracardiac pressures were lower after digitalization than before. During exercise, at identical work loads, the heart rate was lower, the stroke volume unchanged, the cardiac output consequently somewhat lower and the intracardiac pressures markedly lower (P <0.001). These results were thought to indicate improved myocardial function in twelve cases (57 %) whereas in nine cases (43 %) no improvement was noted. The occurrence of *angina pectoris* was prevented in nine (53 %) out of seventeen patients. This favorable effect of the drug was usually associated with improved myocardial function.

C. There was no significant hemodynamic difference between patients with and those

without *clinically recognized myocardial* infarctions. Patients in *functional Class* I—II had higher mean stroke volumes and lower mean intracardiac pressures during exercise than those belonging to functional Class III—IV. A significant difference was only noted between the PCV pressures ($P < 0.01$). *The degree of previous physical activity* had no significant influence upon the hemodynamics during exercise. The hemodynamic variables were not correlated to the *electrocardiographic changes* occurring during exercise. This was probably due to the selection of the material. Patients with *exercise tolerance* of ≥ 600 kpm/min. had significantly larger stroke volumes and lower ventricular filling pressures than the patients with exercise tolerance < 600 kpm/min. ($P < 0.01$). Signs of left ventricular failure during light exercise were only observed in cases with exercise tolerance < 600 kpm/min. This emphasized the greater importance of the exercise tolerance when compared with the functional classification system as a test for evaluation of patients with coronary heart disease. There was no correlation between the hemodynamic data obtained during exercise, and the different *angiographic changes* in the coronary arteries graded with the present grading system. This could be explained by the fact that the coronary angiography did not reflect the coronary blood flow during exercise and, therefore, could not be used as a quantitative variable to measure coronary insufficiency or myocardial hypoxia.

The cardiac performance during light to moderate exercise in the present material was limited by angina pectoris in twenty six patients (72 %). Fourteen of these patients (39 % of the whole material) had elevated ventricular filling pressures suggestive of myocardial insufficiency while twelve (33 %) had only a more or less hypokinetic circulation. Out of the ten patients in whom angina pectoris was not precipitated only one had elevated pressures and a small stroke volume, five (14 %) had only a hypokinetic circulation and in four (11 %) no hemodynamic abnormalities that could limit their cardiac performance were found during light to moderate exercise.

X

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XI

APPENDIX

Table 1 Some anthropometric and clinical data for 38 male patients with coronary heart disease

Case No	Clinical diagnosis	Age, years	Height cm	Body weight, kg	BSA m^2	Relative heart volume ml/m^2 BSA	Total heart volume ml	Total hemoglobin, g
1	St p inf.myoc.	40	176	72	1.87	360	1100	645
2	St p.inf.myoc	53	174	63	1.74	320	610	710
3	St p.inf.myoc.	51	180	83	2.04	460	1070	670
4	St p.inf.myoc.	48	161	70	1.73	410	650	565
5	St.p.inf.myoc.	53	174	66	1.81	520	1110	660
6	St p.inf.myoc.	48	177	75	1 91	500	—	960
7	Ang pect.	55	176	78	1 94	420	—	770
8	Ang pect.	55	164	69	1.74	410	780	715
9	St.p.inf.myoc.	44	172	70	1.82	530	—	690
10	Ang.pect.	55	174	71	1.85	420	810	500
11	St.p.inf.myoc.	55	179	82	2.00	370	870	535
12	Ang.pect.	47	181	89	2.09	400	870	680
13	St.p.inf.myoc.	47	172	72	1.84	430	790	735
14	Ang pect.	56	165	67	1.73	370	770	570
15	Ang pect.	51	181	87	2.06	400	820	760
16	Ang pect.	58	167	68	1.76	530	870	520
17	St.p.inf.myoc.	56	177	78	1 95	490	1150	760
18	St.p.inf.myoc	57	175	95	2.09	490	—	965
19	St p.inf.myoc.	54	171	74	1.85	500	870	870
20	St.p.inf.myoc	41	172	68	1.79	410	860	540
21	St.p.inf.myoc.	40	172	66	1.77	380	830	800
22	Ang pect	53	172	64	1.76	310	720	615
23	St p.inf.myoc.	51	189	96	2.26	360	770	865
24	St.p.inf myoc.	52	171	71	1 84	420	790	910
25	Ang.pect	52	174	69	1.84	400	740	800
26	St.p inf myoc.	58	170	64	1.74	420	900	635
27	Ang pect	49	180	73	1 94	460	890	815
28	A g pect.	50	165	73	1.80	400	710	500
29	A g pect	45	175	101	2.14	470	1150	880
30	Ang pect.	42	186	80	2.06	360	—	855
31	A g pect.	56	164	64	1.68	450	820	720
32	Ang pect.	52	172	67	1 80	440	—	660
33	St.p.inf.myoc.	53	169	68	1.80	410	790	670
34	Ang pect.	44	174	71	1.86	410	800	740
35	Ang.pect.	50	183	93	2.15	420	—	820
36	A g.pect.	56	184	79	2.04	480	1000	805
37	St.p.inf.myoc.	49	164	72	1.78	300	700	640
38	Ang pect.	42	177	71	1.88	330	680	720

Case No.	Resting blood pressure, mm Hg		Phys. act. Group I—III	Functional class I—IV	Time interval since last infarction, months	No. of infarctions	Duration of sympt., months	Type of symptoms		
								Angina pectoris		Effort dyspnoea
	S	D						Anxiety	Exercise	
1	125	85	I	III	11	4	84	+	+	—
2	150	90	II	III	13	2	84	+	+	+
3	170	95	I	II	8	1	18	—	+	+
4	140	90	II	II	21	3	22	—	+	+
5	140	80	I	II	14	3	108	+	+	—
6	125	85	I	II	12	1	20	—	+	+
7	130	85	III	I		0	33	—	+	—
8	125	75	II	III		0	144	+	+	—
9	125	85	III	II	24	1	36	—	+	—
10	125	90	III	II		0	12	—	+	—
11	160	85	I	III	>100	1	72	+	+	—
12	155	100	II	I		0	36	—	+	—
13	150	95	I	IV	38	1	72	+	+	—
14	145	80	I	III		0	24	—	+	—
15	120	80	I	II		0	7	—	+	—
16	120	90	I	II		0	72	—	+	—
17	150	85	III	III	6	1	10	+	+	+
18	150	85	I	I	10	1	12	—	+	—
19	120	90	III	III	6	1	12	—	+	—
20	150	90	I	I	14	1	14	—	+	—
21	145	75	III	I	6	1	6	—	+	—
22	160	100	II	II		0	6	—	+	+
23	120	80	III	II	15	1	15	+	+	—
24	140	90	III	II	6	1	18	—	+	—
25	130	90	II	II		0	108	—	+	—
26	155	105	III	III	24	1	72	+	+	—
27	130	80	II	II		0	4	—	+	—
28	155	80	I	III		0	84	+	+	+
29	165	95	III	II		0	18	+	+	+
30	125	70	II	II		0	18	—	+	—
31	160	95	III	III		0	36	—	+	+
32	140	90	III	III		0	48	+	+	—
33	135	80	III	III	24	1	24	+	+	+
34	130	90	III	II		0	144	—	+	—
35	130	75	II	II		0	228	—	+	+
36	135	95	I	II		0	24	+	+	+
37	155	90	I	III	11	1	12	—	+	+
38	130	85	I	II		0	7	+	+	—

Table 2. Some anthropometric, clinical and functional data for 11 men used as control subjects.

Case No.	Diagnosis	Age, years	Height, cm	Body weight, kg	BSA, m	Relative heart volume, ml/m² BSA	Total heart volume, ml	Total hemoglobin, g	Resting blood pressure, mm Hg		Phys. acti- vity Group I—III	Exercise tolerance test	
									S	D		kpm/min	Heart rate, beats/min
39	Funct. murm r	39	184	74	1.97	430	860	745	135	80	I	600	160
40	Obs. Chest pain	41	179	98	2.18	430	950	740	140	90	I	900	165
41	Volunteer	51	173	61	1.72	440	950	750	130	75	III	1200	170
42	Volunteer	53	171	87	1.98	500	1000	880	150	90	II	900	160
43	Volunteer	30	180	73	1.93	440	870	795	140	80	III	1200	168
44	Volunteer	52	174	75	1.89	320	830	675	130	80	III	1500	168
45	Volunteer	51	176	97	2.14	440	940	920	140	90	II	1200	149
46	Obs. PAT	50	164	69	1.76	470	880	665	140	90	III	900	142
47	Volunteer	56	180	86	2.06	500	1070	700	130	95	II	900	132
48	Volunteer	53	187	92	2.20	450	980	—	120	80	II	1200	158
49	Funct. m murmur	49	174	74	1.88	410	870	620	150	85	III	900	173

Table 3 The incidence of significant electrocardiographic changes at rest, during and 3 minutes after exercise in 38 male patients with coronary heart disease. Q= Significant initial QRS vector abnormality (Blackburn et al 1960) VEB=ventricular ectopic beat.

Case No.	Clinical diagnosis	Q	Rest			Exercise		
			S-T depr ≧1 mm	T wave inversion	VEB	S-T depr ≧1 mm	T wave changes	VEB
1	St.p.inf.myoc.	+			+			+
2	St.p.inf.myoc.	+				+		
3	St.p.inf.myoc.							
4	St.p.inf.myoc.			+		+		
5	St.p.inf.myoc.	+		+		+		
6	St.p.inf.myoc.	+		+				
7	Ang. pect.	+				+	+	
8	Ang. pect.					+		
9	St.p.inf.myoc.	+						
10	Ang. pect.					+		
11	St.p.inf.myoc.	+	+			+	+	
12	Ang. pect.	+	+			+		+
13	St.p.inf.myoc.	+	+	+			+	+
14	Ang. pect.							
15	Ang. pect.					+		
16	Ang. pect.		+			+		
17	St.p.inf.myoc.		+	+		+		
18	St.p.inf.myoc.	+						
19	St.p.inf.myoc.	+						+
20	St.p.inf.myoc.	+		+			+	
21	St.p.inf.myoc.	+				+		+
22	Ang. pect.					+		
23	St.p.inf.myoc.		+		+	+		
24	St.p.inf.myoc.							
25	Ang. pect.					+		
26	St.p.inf.myoc.	+	+	+				+
27	Ang. pect.					+		
28	Ang. pect.	+	+	+		+		
29	Ang. pect.					+		
30	Ang. pect.			+		+		
31	Ang. pect.	+	+			+	+	
32	Ang. pect.	+				+		
33	St.p.inf.myoc.		+			+		
34	Ang. pect.					+		
35	Ang. pect.			+		+	+	
36	Ang. pect.	+	+			+		
37	St.p.inf.myoc.					+		+
38	Ang. pect.		+			+		

Case No.	3 min after exercise			Location of Q-wave, leads			Location of S-T changes, leads		
	S-T depr ≧1 mm	T-wave changes	VEB	I, V_5-V	V_L V_5-V	II, III, V_F	I, V_5-V	aV_L V_5-V	II, III, V_F
1			+	+	+				
2	+				+	+		+	+
3									
4	+	+						+	
5		+			+	+	+	+	+
6		+			+	+			
7	+				+	+		+	+
8	+							+	
9	+		+			+		+	+
10	+						+	+	
11	+	+				+		+	+
12	+					+		+	+
13						+	+	+	+
14	+	+						+	
15							+	+	
16	+						+	+	+
17	+	+						+	+
18				+	+				
19						+			
20		+		+					
21						+		+	+
22	+							+	
23								+	
24	+						+	+	
25	+							+	+
26				+			+	+	
27	+						+	+	
28	+					+		+	+
29	+						+	+	
30	+	+						+	+
31	+	+		+	+		+	+	+
32	+	+		+			+	+	
33	+	+						+	+
34	+	+						+	
35		+						+	
36					+			+	
37	+						+		
38	+						+	+	

Table 4 Clinical diagnosis, degree of previous physical activity functional capacity according to the N.Y.H.A. classification system and results from exercise tolerance tests in 38 male patients with coronary heart disease.

Case No.	Clinical diagnosis	Phys. activity Group I—III	Functional class I—IV	Exercise tolerance test			
				kpm/min	Heart rate, beats/min	Symptoms	
						Ang. pect.	Dyspn.
1	St.p.inf.myoc.	I	III	250	107	+	—
2	St.p.inf.myoc.	II	III	200	134	+	—
3	St.p.inf.myoc.	I	II	600	120	—	—
4	St.p.inf.myoc.	II	II	500	142	+	—
5	St.p.inf.myoc.	I	II	100	122	+	—
6	St.p.inf.myoc.	I	II	200	118	+	+
7	Ang. pect.	III	I	600	119	+	—
8	Ang. pect.	II	III	300	104	+	—
9	St.p.inf.myoc.	III	II	400	118	+	—
10	Ang. pect.	III	II	300	111	+	—
11	St.p.inf.myoc.	I	III	200	120	+	—
12	Ang. pect.	II	I	600	143	—	—
13	St.p.inf.myoc.	I	IV	100	103	+	—
14	Ang. pect.	I	III	400	108	+	—
15	Ang. pect.	I	II	500	140	+	—
16	Ang. pect.	I	II	300	104	+	—
17	St.p.inf.myoc.	III	III	200	87	+	—
18	St.p.inf.myoc.	I	I	400	112	+	—
19	St.p.inf.myoc.	III	III	200	106	+	—
20	St.p.inf.myoc.	I	I	600	120	—	—
21	St.p.inf.myoc.	III	I	500	105	—	+
22	Ang. pect.	II	II	400	132	+	+
23	St.p.inf.myoc.	III	II	600	138	+	+
24	St.p.inf.myoc.	III	II	600	138	+	—
25	Ang. pect.	II	II	400	115	+	—
26	St.p.inf.myoc.	III	III	300	92	+	—
27	Ang. pect.	II	II	800	140	+	—
28	Ang. pect.	I	III	200	90	+	—
29	Ang. pect.	III	II	900	142	+	+
30	Ang. pect.	II	II	400	117	+	—
31	Ang. pect.	III	III	200	107	+	—
32	Ang. pect.	III	III	200	102	+	—
33	St.p.inf.myoc.	III	III	300	133	+	—
34	Ang. pect.	III	II	600	142	+	—
35	Ang. pect.	II	II	800	156	+	+
36	Ang. pect.	I	II	400	130	+	—
37	St.p.inf.myoc.	I	III	200	147	+	—
38	Ang. pect.	I	II	400	130	+	—

Table 5 Primary hemodynamic data obtained during right heart catheterization at rest and during exercise for 36 male patients with coronary heart disease. Ced.=intravenous injection of Cedilanid® one hour before the study. NaCl=intravenous injection of saline one hour before the study. R=rest. E=exercise. V_{O_2}=oxygen uptake. O_2—cap.=oxygen capacity of arterial blood. S_{O_2}=oxygen

Case No	Experimental conditions		Time, min	Work load kpm/min	Heart rate, beats/min	V_{O_2} ml/min, STPD	O_2-cap ml/100 ml	S_{O_2} %	a-v O_2 diff ml/l	$\dot{Q}$ l/min	Strok. vol. ml/beat
1		R			72	221	15.9	94.0	32	6.9	96
		E	5	150	108	608	15.9	97.7	66	9.2	85
	Ced. 1.2 mg	R			72	238	15.9	94.9	33	7.2	100
		E	5	150	105	610	16.0	99.0	64	9.5	[illegible]
2		R			81	268	14.3	98.4	43	6.2	77
		E	6	200	134	754	14.9	93.7	71	10.6	79
	Ced. 1.2 mg	R			93	251	13.5	97.2	42	6.0	65
		E	6	200	138	782	13.9	96.1	78	10.0	72
4		R			66	263	18.1	99.6	61	4.3	65
		E	7	300	123	984	18.2	97.5	101	9.7	79
	Ced. 1.2 mg	R			62	221	18.0	100.5	56	3.9	63
		E	7	300	126	992	18.7	100.5	116	8.6	68
5		R			75	181	16.8	93.9	41	4.4	59
		E	6	100	144	657	16.3	99.7	105	6.3	44
	Ced. 1.2 mg	R			69	212	15.5	97.5	37	5.7	83
		E	6	100	116	630	15.9	96.5	90	7.0	60
6		R			75	303	17.6	95.6	50	6.1	81
		E	6	250	114	1050	18.3	96.9	117	9.0	79
	Ced. 1.2 mg	R			81	259	17.3	94.6	39	6.6	81
		E	6	250	117	958	18.7	94.4	111	8.6	74
9		R			86	260	15.9	96.0	38	6.8	79
		E	5	200	114	801	16.7	94.0	75	10.7	94
	Ced. 1.2 mg	R			84	281	15.7	96.6	37	7.6	90
		E	5	200	108	773	16.3	93.9	73	10.6	98
11		R			78	240	14.3	96.1	49	4.9	63
		E	5	200	118	703	14.6	97.9	104	6.8	58
	Ced. 1.2 mg	R			74	245	13.8	98.8	42	5.8	78
		E	5	200	114	668	14.9	97.6	111	6.0	33
13		R			93	245	19.0	92.4	43	5.7	61
		E	5	100	111	620	18.6	93.2	67	9.3	84
	Ced. 1.6 mg	R			84	47	18.4	92.4	38	6.5	77
		E	5	100	96	635	18.7	95.3	76	8.4	88
17		R			64	266	18.8	93.3	47	5.7	89
		E	6	100	92	858	18.2	94.4	79	10.9	118
	Ced. 1.6 mg	R			58	279	18.1	94.1	45	6.2	107
		E	6	100	80	766	18.9	96.1	84	9.1	114

saturation of arterial blood. a-vO_2 diff.=arteriovenous oxygen difference between systemic and pulmonary arterial blood. $\dot{Q}$=cardiac output. S=systolic pressure. D=diastolic pressure. ed=end diastolic pressure. $\bar{P}$=electrically integrated mean pressure.

Case No.	Blood pressures, mm Hg									Vascular resistance, dyn sec cm⁻⁵		Symptoms during exercise
	RV		PA			PCV	BA					
	S	ed	S	D	$\bar{P}$	P	S	D	P	Pulm.	Systemic	
1	25	8	22	11	17	10	—	—	—	80	—	
	—	—	32	21	25	—	120	96	106	—	920	No pain
	—	—	19	8	13	8	111	85	100	60	1110	
	—	—	33	19	27	20	—	—	—	60	—	No pain
2	23	5	20	8	13	8	136	87	108	60	1390	
	45	13	44	22	30	21	153	102	117	70	880	Pain
	—	—	19	8	12	4	142	89	106	110	1410	
	37	3	36	14	21	10	173	104	128	90	1020	No pain
4	32		27	9	15	7	159	90	117	150	2180	
	50	3	39	12	20	3	183	104	143	140	1180	No pain
	39	2	30	10	16	1	161	86	114	310	2340	
	53	1	42	13	21	4	193	107	135	160	1260	No pain
5	26	2	26	13	18	10	127	73	94	150	1710	
	62	18	62	45	54	41	156	94	113	170	1430	Pain
	19	2	19	6	10	3	125	66	84	100	1180	
	56	8	45	27	33	23	162	90	120	140	1370	Pain
6	21	9	20	9	13	9	96	65	76	50	1000	
	49	24	49	31	37	30	126	84	105	60	930	No pain
	22	6	18	8	12	6	111	70	83	70	1010	
	54	19	50	28	35	26	143	87	108	80	1000	No pain
9	23	2	22	10	14	7	153	93	120	80	1410	
	4	4	48	26	31	17	200	109	152	100	1140	Pain
	23	4	23	10	16	5	160	93	117	120	1230	
	40	5	42	22	29	10	202	110	148	140	1120	No pain
11	28	6	25	11	17	11	168	99	117	100	1910	
	65	21	58	37	47	41	200	110	143	70	1640	Pain
	22	3	20	7	10	6	177	87	116	60	1600	
	68	19	67	44	54	40	196	104	141	190	1880	Pain
13	19	5	19	10	13	8	167	93	107	100	1500	
	43	8	34	25	30	23	186	103	140	60	1200	Pain
	22	3	22	10	13	6	152	80	103	90	1270	
	30	4	26	8	16	8	200	102	138	80	1310	No pain
17	29	7	26	11	19	7	168	92	124	170	1740	
	48	13	47	29	34	19	200	104	136	120	1000	Pain
	19	1	19	5	9	2	154	81	106	90	1370	
	32	3	25	11	18	6	188	108	136	110	1200	No pain
24	22	6	19	11	13	8	190	114	[illegible]	[illegible]	[illegible]	
	41	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

Table 5 Primary hemodynamic data obtained during right heart catheterization at rest and during exercise for 36 male patients with coronary heart disease Ced.=intravenous injection of Cedilanid® one hour before the study NaCl=Intravenous injection of saline one hour before the study R=rest. E=exercise V_{O_2}=oxygen uptake O_2—cap.=oxygen capacity of arterial blood. S_{O_2}=oxygen

Case No.	Experimental conditions		Time min	Work load, kpm/min	Heart rate, beats/min	V_{O_2}, ml/min STPD	O_2-cap ml/100 ml	S_{O_2} /	a v O_2 diff ml/l	$\dot{Q}$ l/m	Stroke volume ml/beat
1		R			72	221	15 9	94.0	32	6 9	96
		E	5	150	108	608	15 9	97.7	66	9.2	85
	Ced. 1.2 mg	R			72	238	15.9	94.9	33	7.2	100
		E	5	150	105	610	16.0	99.0	64	9.5	90
2		R			81	268	14.3	98 4	43	6.2	77
		E	6	200	134	754	14 9	93.7	71	10.6	79
	Ced. 1.2 mg	R			93	251	13.5	97.2	42	6 0	65
		E	6	200	138	782	13 9	96 1	78	10.0	72
4		R			66	263	18.1	99.6	61	4.3	65
		E	7	300	123	984	18.2	97.5	101	9.7	79
	Ced. 1.2 mg	R			62	221	18.0	100.5	56	3.9	63
		E	7	300	126	992	18.7	100.5	116	8.6	68
5		R			75	181	16.8	93.9	41	4 4	59
		E	6	100	144	657	16.3	99.7	105	6.3	44
	Ced. 1.2 mg	R			69	212	15.5	97.5	37	5.7	83
		E	6	100	116	630	15.9	96.5	90	7.0	60
6		R			75	303	17.6	95 6	50	6 1	81
		E	6	250	114	1050	18.3	96.9	117	9 0	79
	Ced. 1.2 mg	R			81	259	17.3	94.6	39	6.6	81
		E	6	250	117	958	18.7	94 4	111	8.6	74
9		R			86	260	15 9	96.0	38	6.8	79
		E	5	200	114	801	16.7	94.0	75	10.7	94
	Ced. 1.2 mg	R			84	81	15.7	96.6	37	7.6	90
		E	5	200	108	773	16.3	93 9	73	10.6	98
11		R			78	240	14.3	96 1	49	4 9	63
		E	5	200	118	703	14.6	97 9	104	6.8	58
	Ced. 1.2 mg	R			74	245	13.8	98 8	42	5.8	78
		E	5	100	114	668	14 9	97.6	111	6.0	53
13		R			93	245	19 0	92.4	43	5.7	61
		E	5	100	111	620	18.6	93.2	67	9 3	84
	Ced. 1.6 mg	R			84	247	18.4	92.4	38	6 5	77
		E	5	100	96	635	18.7	95.3	76	8 4	88
17		R			64	266	18.8	93.3	47	5.7	89
		E	6	100	92	858	18.2	94 4	79	10.9	118
	Ced. 1.6 mg	R			58	279	18.1	94 1	45	6.2	107
			[illegible]	[illegible]	[illegible]	[illegible]	18.8	96 1	84	9 1	114

Case No.	Blood pressures, mm Hg									Vascular resistance, dyn sec cm⁻⁵		Symptoms during exercise
	RV		PA			PCV	BA					
	S	ed	S	D	$\bar{P}$	$\bar{P}$	S	D	$\bar{P}$	Pulm.	Systemic	
7	19	2	18	7	11	5	118	59	80	80	1000	
	36	5	34	18	25	14	136	74	103	70	640	No pain
	22	2	19	10	13	4	112	52	76	100	860	
	32	3	35	14	24	10	154	70	97	90	600	No pain
8	23	3	23	9	14	9	143	84	100	80	1510	
	—	—	43	19	29	19	—	—	129	80	1080	Pain
	20	1	20	6	11	9	141	85	111	30	1390	
	41	4	39	15	25	14	167	92	126	80	960	Pain
15	20	3	18	7	13	3	160	88	120	80	930	
	59	7	54	31	43	30	—	—	—	90	—	Pain
	17	0	17	3	9	2	155	86	110	60	990	
	—	—	56	37	45	35	—	—	—	70	—	Pain
22	20	3	17	5	9	9	146	76	108	—	1880	
	50	6	45	25	33	—	210	108	154	—	1420	Pain
	20	3	18	5	9	2	144	78	100	110	1570	
	48	5	38	20	31	21	205	98	140	100	1440	Pain
25	21	1	17	5	10	3	128	77	100	100	1380	
	58	8	48	33	40	30	184	120	146	70	1070	Pain
	15	1	14	3	6	1	128	67	93	60	1160	
	45	3	42	15	23	15	—	—	—	80	—	No pain
27	19	6	19	10	14	6	158	81	106	110	1440	
	—	—	—	—	26	17	192	100	146	70	1130	No pain
	33	7	33	17	26	20	220	100	158	30	880	Pain
	19	3	17	8	12	7	182	94	142	70	1860	
	—	—	—	—	19	9	212	103	140	70	1040	No pain
	33	6	27	16	21	12	235	110	158	90	840	N pain
29	—	—	30	17	22	11	148	91	110	150	1490	
	—	—	52	30	38	20	192	100	142	140	1110	Pain
	24	3	24	12	15	6	129	84	98	120	1290	
	34	3	25	13	16	4	148	84	116	100	970	No pain
31	22	4	20	8	13	7	127	78	97	90	1520	
	70	13	71	36	53	38	189	104	132	160	1490	Pain
	22	2	19	8	12	4	153	83	106	130	1730	
	67	11	58	32	43	31	206	109	144	130	1520	Pain
32	25	4	23	8	11	6	175	108	128	60	1440	
	—	—	48	25	31	—	205	123	169	—	970	Pain
	17	1	17	6	10	1	168	114	138	80	1280	
	—	—	23	11	15	10	220	128	174	30	1160	No pain

Table 3 (continued)

Case No	Experimental conditions		Time, min	Work load kpm/min	Heart rate, beats/min	V_{O_2} ml/min, STPD	O_2-cap ml/100 ml	S_{O_2} %	a v O_2 diff ml/l	$\dot{Q}$ l/min	Stroke vol me, ml/beat
7		R			58	251	14 4	94.2	39	6.4	110
		E	6	250	90	877	14 4	93.7	70	12.5	139
	Ced. 1.2 mg	R			57	255	14.3	93.2	36	7 1	125
		E	6	250	87	889	14 9	93.6	69	12.9	148
8		R			66	205	17.2	96.2	39	5.3	80
		E	3	200	106	730	17.8	96.2	76	9.6	91
	Ced. 1.2 mg	R			75	199	16.9	94.2	31	6 4	85
		E	3	200	108	828	17.7	95.8	79	10.5	97
15		R			96	289	19.2	93.8	28	10.3	107
		E	4	400	138	1107	19.3	96.9	99	11.2	81
	Ced. 1.6 mg	R			108	302	18.8	94.0	34	8.9	82
		E	4	400	140	1107	19.9	95.3	103	10.7	76
22		R			87	184	18.7	95 1	40	4.6	53
		E	7	250	120	817	18.7	95 1	94	8.7	73
	Ced. 1.2 mg	R			80	168	18.1	95.8	33	5 1	64
		E	7	250	117	778	19.2	96.0	100	7.8	67
25		R			69	250	17.9	93 4	43	5.8	84
		E	6	230	118	980	18.8	95 4	90	10.9	92
	Ced. 1.6 mg	R			66	257	17 9	94.3	40	6 4	97
		E	6	250	100	924	18.8	95.2	97	9.5	95
27		R			62	247	18.0	95.9	42	5 9	95
		EI	6	250	92	954	18.7	96.0	93	10.3	112
		EII	6	500	112	1370	19.2	96.8	96	14.3	128
	Ced. 1.6 mg	R			60	274	18.2	97.7	45	6.1	102
		EI	6	250	86	1006	18.9	96.3	93	10.8	126
		EII	6	500	108	1483	19.5	96.7	98	15 1	140
29		R			76	281	18.2	97.8	48	5 9	78
		E	6	250	104	1035	18.9	97 4	101	10.2	98
	Ced. 1.6 mg	R			90	301	18 1	97.0	49	6 1	68
		E	6	250	104	977	18.7	97 1	101	9.7	93
31		R			80	233	18.8	96.8	46	5 1	64
		E	6	100	108	689	19 4	96.6	95	7.3	68
	Ced. 1.2 mg	R			78	239	18.9	93 9	49	4 9	63
		E	6	100	99	656	20.0	95.6	86	7.6	77
32		R			78	248	17.3	95 4	35	7 1	91
		E	3	100	114	798	17.7	96.0	57	14.0	123
	Ced. 1.6 mg	R			84	250	17.3	96.3	29	8.6	102
		E	3	100	96	638	17.8	96.0	53	12.0	125

Case No.	Blood pressures, mm Hg									Vascular resistance, dyn sec cm⁻⁵		Symptoms during exercise
	RV		PA			PCV	BA					
	S	ed	S	D	P	P	S	D	$\bar{P}$	Pulm.	Systemic	
18	32	6	30	14	21	—	148	84	108	—	1440	
	50	8	50	23	36	—	188	85	118	—	840	
	—	—	32	7	15	6	166	79	104	110	1300	
	49	9	46	26	35	23	164	93	132	80	880	
19	23	4	22	9	14	9	157	100	126	70	1770	
	38	6	39	25	30	19	184	104	130	110	1330	Pain
	22	5	22	10	16	—	142	94	110	—	2000	
	37	—	38	23	28	—	142	108	126	—	1400	Pain
24	28	5	27	11	15	8	—	—	—	80	—	
	41	5	37	14	25	7	170	83	127	130	950	Pain
	25	4	24	10	13	—	130	73	97	—	1550	
	39	5	35	15	25	7	162	86	118	150	950	Pain
10	16	2	13	6	10	3	150	92	105	110	1650	
	—	—	31	8	15	3	—	—	—	70	—	Pain
	14	2	16	7	9	5	134	91	111	70	1810	
	—	—	26	8	14	7	—	—	—	50	—	Pain
12	19	2	19	7	10	7	196	115	150	30	1520	
	—	—	34	16	24	17	223	125	162	50	1180	
	43	5	—	—	36	29	240	130	170	40	1010	Pain
	13	2	13	4	7	6	153	100	126	10	1770	
	—	—	37	17	23	17	226	126	158	40	1060	
	—	—	46	29	35	30	235	156	192	30	1340	Pain
14	25	6	22	11	15	8	143	76	100	90	1310	
	58	16	54	32	43	35	180	91	120	70	1090	Pain
	24	6	25	—	17	13	133	69	95	50	1270	
	57	16	53	33	42	33	180	106	123	80	1040	Pain
16	28	7	29	11	15	8	164	91	116	110	1890	
	48	7	43	18	24	12	—	—	—	110	—	
	59	13	57	26	38	25	194	109	131	—	—	Pain
	27	5	27	11	16	9	160	86	122	110	1990	
	—	—	42	19	25	14	194	93	128	100	1160	
	58	11	56	27	39	23	192	97	140	—	—	Pain
30	22	6	20	10	13	10	128	72	98	30	990	
	—	—	34	22	27	19	162	79	114	50	770	Pain
	19	3	22	11	14	7	114	70	84	90	1030	
	—	—	35	18	26	15	160	88	114	80	870	Pain
35	26	7	22	12	16	6	136	69	97	110	1090	
	33	3	28	20	23	10	146	81	104	70	800	
	24	3	25	15	16	7	125	73	91	100	980	
	36	4	34	17	22	9	154	83	104	90	680	

Table 5 (continued)

Case No	Experimental conditions		Time, min	Work load kpm/min	Heart rate, beats/min	$\dot{V}_{O_2}$ ml/min, STPD	O_2-cap ml/100 ml	S_{O_2} %	a-v O_2 diff ml/l	Q l/min	Stroke vol ml/beat
18		R			78	276	18.2	100.0	46	6.0	77
		E	6	300	120	1032	19.2	97.8	92	11.2	93
	NaCl, 6 ml	R			84	295	18.3	97.8	46	6.4	76
		E	6	300	118	1034	18.2	94.8	86	12.0	102
19		R			88	239	17.0	94.3	42	5.7	65
		E	6	150	123	671	17.5	96.8	86	7.8	63
	NaCl 6 ml	R			81	237	17.2	96.4	54	4.4	54
		E	6	150	120	630	17.3	94.7	87	7.2	60
4		R			65	306	18.0	93.0	43	1	109
		E	5	250	93	879	18.6	91.0	82	10.7	115
	NaCl 6 ml	R			60	225	17.8	92.0	45	5.0	83
		E	5	250	93	853	18.1	92.0	86	9.9	106
10		R			82	199	15.0	96.2	39	5.1	62
		E	6	300	118	1125	15.5	94.6	87	12.9	109
	NaCl 6 ml	R			88	188	14.1	96.0	38	4.9	56
		E	6	300	126	1037	15.7	95.0	91	11.4	90
12		R			78	283	14.9	96.	36	7.9	101
		EI	5	250	122	917	15.9	96.7	83	11.0	90
		EII	5	500	144	1305	15.9	96.8	97	13.5	94
	NaCl, 6 ml	R			90	298	15.4	99.3	52	5.7	63
		EI	5	250	126	939	15.4	96.4	79	11.9	94
		EII	5	500	145	1246	16.3	98.8	108	11.5	79
14		R			74	256	17.4	94.7	42	6.1	82
		E	5	200	102	825	17.8	96.7	94	8.8	86
	N Cl, 6 ml	R			78	239	16.9	95.8	40	6.0	77
		E	5	200	102	804	16.9	95.6	85	9.5	93
16		R			63	231	17.0	97.1	47	4.9	78
		EI	5	100	90	573	17.4	98.3	68	8.4	93
		EII	4	200	102	—	—	—	—	—	—
	NaCl 6 ml	R			63	220	16.7	98.8	45	4.9	78
		EI	5	100	90	625	16.9	100.0	71	8.8	98
		EII	4	200	101	—	—	—	—	—	—
30		R			66	372	17.0	96.1	47	7.9	120
		E	6	250	98	1086	18.1	97.6	92	11.8	120
	NaCl, 6 ml	R			68	325	17.0	96.2	50	6.5	96
		E	6	250	104	928	17.6	97.3	88	10.5	101
35		R			84	319	18.5	94.9	45	7.1	85
		E	5	250	123	1128	18.8	94.9	79	14.3	116
	NaCl, 6 ml	R			96	310	18.6	94.8	42	7.4	77
		E	5	250	111	966	19.0	94.8	79	12.2	110

Case No.	Blood pressures, mm Hg									Vascular resistance, dyn sec cm^{-5}		Symptoms during exercise
	RV		PA			PCV	BA					
	S	ed	S	D	$\bar{P}$	$\bar{P}$	S	D	$\bar{P}$	Pulm.	Systemic	
3	—	—	22	12	15	7	172	111	124	70	1120	
	—	—	34	20	26	12	190	126	158	80	930	No pain
20	27	6	21	9	13	7	172	88	112	80	1470	
	—	—	32	14	22	9	220	111	148	110	1300	
	46	3	32	—	21	7	220	98	146	90	970	No pain
21	20	3	15	5	9	3	134	85	108	70	1310	
	42	0	27	8	14	4	152	82	112	70	830	No pain
23	24	4	18	8	12	10	136	75	108	30	1460	
	24	0	26	11	17	9	168	85	112	70	910	No pain
28	19	5	19	10	14	—	156	71	103	—	1620	
	—	—	—	—	48	35	200	106	152	160	1870	Pain
34	23	5	22	9	14	7	138	86	106	90	1440	
	—	—	24	14	16	9	159	90	118	60	930	
	34	2	26	12	18	6	160	84	108	—	—	Pain

Table 5 (continued)

Case No.	Experimental conditions	Time, min	Work load, kpm/min	Heart rate beats/min	$\dot{V}_{O_2}$ ml/min, STPD	O_2-cap ml/100 ml	S_{O_2} %	a v O_2 diff ml/l	Q l/min	Strok volum ml/beat
3	R			80	309	15.7	96.7	35	8.8	110
	E	12	250	105	914	16.2	95.6	67	13.6	130
20	R			66	256	17.8	97.9	42	6.1	92
	EI	6	250	104	877	18.7	97 4	96	9 1	88
	EII	6	500	134	1327	19.3	98 0	111	12.0	90
21	R			69	257	20.0	96.2	39	6.6	96
	E	6	350	116	1054	20.5	95.3	98	10.8	93
23	R			69	301	19.6	94 4	51	5 9	86
	E	5	250	96	972	20.3	92.5	99	9.8	102
28	R			76	309	16.6	96.1	61	5 1	67
	E	5	100	110	672	16.7	94.6	103	6.5	59
34	R			78	266	18.7	96.0	45	5.9	76
	EI	6	250	105	765	19 1	95 9	80	9.6	91
	EII	6	500	126	—	—	—	—	—	—

Case No.	Blood pressures, mm Hg									Vascular resistance, dyn sec cm	
	RV		PA			PCV	BA				
	S	ed	S	D	$\bar{P}$	$\bar{P}$	S	D	$\bar{P}$	Pulm.	Systemic
39	23	5	23	9	14	9	123	72	99	40	730
	—	—	30	15	21	11	—	—	—	60	—
	30	6	28	13	20	11	143	84	105	40	500
40	24	4	22	9	13	8	133	93	120	40	1030
	—	—	39	17	25	11	177	100	127	90	830
	—	—	40	16	21	9	177	93	127	60	600
41	22	5	18	9	13	9	131	74	100	40	990
	—	—	36	16	23	16	156	76	105	50	740
	44	7	34	13	21	11	176	82	106	50	570
42	28	3	24	9	13	5	188	92	134	60	1010
	—	—	38	16	24	8	246	104	150	80	790
	—	—	47	14	26	5	256	109	165	100	820
43	23	2	21	7	11	7	160	88	109	40	1180
	—	—	23	10	16	8	—	—	114	40	640
	41	4	29	10	16	8	—	—	114	40	540
44	19	2	14	6	9	8	115	67	85	10	940
	—	—	32	11	20	11	134	66	88	60	610
	41	4	29	11	19	11	158	68	97	40	500
45	24	4	24	10	15	8	144	93	121	90	1620
	—	—	36	19	26	13	—	—	—	100	—
	40	6	37	17	25	11	171	88	112	80	630
46	17	1	16	5	12	4	136	70	97	110	1360
	—	—	30	9	16	9	—	—	—	50	—
	38	0	32	11	17	9	200	85	120	40	670
47	21	4	23	11	16	11	168	84	116	80	1820
	—	—	38	20	28	20	207	96	140	60	1110
	32	3	35	15	26	15	176	72	116	60	680
48	23	6	23	11	15	11	132	78	106	60	1700
	—	—	44	21	29	—	160	88	112	—	940
	39	5	38	17	24	—	185	97	124	—	780
49	17	2	16	4	9	2	144	85	100	100	1450
	35	0	26	9	16	4	172	88	116	100	920

Table 6 Primary hemodynamic data obtained during right heart catheterization at rest and during exercise for 11 male control subjects. Abbreviations as in table 5

Case No	Experimental conditions	Time, min	Work load, kpm/min	Heart rate beats/min	$\dot{V}_{O_2}$, ml/min, STPD	O_2-cap ml/100 ml	S_{O_2} %	a-v O_2 diff ml/l	Q, l/min	Stroke vol me, ml/beat
39	R			93	302	15.9	95.6	28	10.8	116
	EI	5	150	117	745	16.6	96.4	56	13.3	114
	EII	5	300	132	953	16.2	98.8	57	16.7	127
40	R			70	287	15.7	92.2	31	9.3	133
	EI	6	250	92	923	17.3	92.9	75	12.3	134
	EII	6	500	114	1271	16.9	92.3	75	16.9	148
41	R			62	259	16.3	98.0	32	8.1	131
	EI	6	300	88	984	16.7	96.9	87	11.3	128
	EII	6	600	106	1416	16.9	95.[illegible]	95	14.9	141
42	R			72	317	17.1	93.0	30	10.6	147
	EI	6	250	108	940	18.0	90.7	62	15.2	141
	EII	6	500	132	1482	18.7	88.4	92	16.1	122
43	R			70	282	17.2	97.2	38	7.4	106
	EI	6	250	105	961	18.0	91.7	67	14.3	136
	EII	6	500	114	1257	17.3	91.6	75	16.8	147
44	R			72	233	16.4	93.7	35	7.2	100
	EI	5	300	96	981	17.7	97.3	85	11.5	120
	EII	5	600	117	1519	17.3	95.2	98	15.5	132
45	R			60	257	17.3	95.6	43	6.0	100
	EI	5	250	92	907	18.1	96.7	90	10.1	110
	EII	5	500	100	1346	18.2	95.9	95	14.2	142
46	R			69	262	17.3	96.6	46	5.7	83
	EI	4	250	96	875	15.9	98.7	73	12.0	125
	EII	4	500	110	1288	15.9	98.6	90	14.3	130
47	R			56	210	16.7	96.9	41	5.1	91
	EI	5	250	78	901	17.4	94.6	89	10.1	129
	EII	5	500	93	1302	18.3	96.0	95	13.7	147
48	R			62	238	18.7	96.9	48	5.0	81
	EI	7	250	92	1017	19.3	98.2	107	9.5	103
	EII	8	500	107	1425	19.9	98.1	112	12.7	119
49	R			84	277	18.8	97.6	50	5.5	65
	E	6	300	108	988	19.3	99.2	98	10.1	94

Table 8. Means, standard errors of the means (S.E.) and standard deviations (S.D.) of hemodynamic data at rest and during exercise for 36 patients with coronary heart disease separated after clinical diagnosis. Abbreviations as in table 5.

	Heart rate, beats/min	V_{O_2} ml/min STPD	a-v O_2 diff ml/l	$\dot{Q}$, l/min	Stroke volume, ml/beat	Blood pressures, mm Hg					Vascular resistance dyn sec cm	
						RV		PA	PCV	BA		
						s	ed	$\bar{P}$	$\bar{P}$	$\bar{P}$	P. lm.	Systemic
St.p.inf.myocard.												
Rest												
M	74.5	259.2	44.1	5.97	80.9	24.9	4.8	14.7	7.9	110.9	89	1561
SE	2.05	7.74	1.56	0.232	3.66	0.96	0.55	0.68	0.48	3.95	8.9	88.5
SD	8.69	32.85	6.61	1.069	15.32	3.97	2.25	2.87	1.97	15.81	34.8	354.0
	18	18	18	18	18	17	17	18	17	16	17	16
Exercise												
M	114.5	867.5	89.4	9.81	87.6	46.5	8.9	30.7	19.0	130.1	96	1089
SE	3.38	43.87	3.76	0.417	4.96	2.45	1.82	2.46	3.01	4.80	8.1	59.5
SD	14.33	186.15	15.96	1.769	21.03	9.80	7.27	10.43	12.04	20.38	32.4	25..4
	18	18	18	18	18	16	16	18	16	18	16	18
Ang. pect.												
Rest												
M	74.7	260.4	43.0	6.19	83.6	22.1	4.2	13.3	7.0	109.4	88	1466
SE	2.27	10.68	1.69	0.336	4.21	0.83	0.46	0.72	0.62	4.10	7.6	80.9
SD	9.63	45.30	7.18	1.426	17.85	3.44	1.88	3.07	2.57	17.39	30.2	343.2
	18	18	18	18	18	17	17	18	17	18	16	18
Exercise												
M	112.4	926.9	84.6	10.81	96.8	48.7	8.0	32.6	21.3	137.8	88	1094
SE	3.39	53.02	3.03	0.570	5.13	3.33	1.06	2.48	2.62	5.79	10.4	79.3
SD	14.37	224.94	12.84	2.417	21.76	11.71	3.52	10.51	10.49	22.42	41.5	307.2
	18	18	18	18	18	11	11	18	16	15	16	15

Table 7 Means, standard errors of the means (S.E.) and standard deviations (S.D.) of hemodynamic data at rest and during exercise in 36 male patients with coronary heart disease and 11 male control subjects. Abbreviations as in table 5

		Heart rate beats/min	$\dot{V}_{O_2}$ ml/min STPD	a-vO_2 diff ml/l	$\dot{Q}$ l/min	Stroke volume, ml/beat	Blood pressures, mm Hg					Vascular resistance dyn sec cm^{-5}	
							RV		PA	PCV	BA		
							S	ed	$\bar{P}$	$\bar{P}$	$\bar{P}$	Pulm.	Systemic
Patients													
Rest	M	74.6	259.8	43.6	6.08	82.3	23.5	4.5	14.0	7.4	110.1	88	1511
	SE	1.51	6.50	1.14	0.208	2.76	0.67	0.35	0.50	0.39	2.82	5.8	59.4
	SD	9.04	39.00	6.83	1.247	16.54	3.93	2.06	3.01	2.30	16.43	33.3	346.3
	n	36	35	36	36	36	34	34	36	31	34	33	34
Exercise	M	113.4	897.2	88.0	10.31	92.2	47.4	8.6	31.6	20.1	133.6	92	1092
	SE	2.36	34.28	2.39	0.358	3.60	2.01	1.15	1.73	1.97	3.72	6.5	47.7
	SD	14.18	205.71	14.35	2.147	21.60	10.46	5.96	10.36	11.17	21.35	36.9	274.1
	n	36	36	36	36	36	27	27	36	32	33	32	33
Controls													
Rest	M	70.0	267.6	38.4	7.34	104.8	21.9	3.5	12.7	7.5	107.9	61	1257
	SE	3.24	9.00	2.33	0.642	7.54	0.99	0.47	0.70	0.85	4.17	9.3	107.1
	SD	10.76	29.87	7.74	2.131	25.00	3.27	1.57	2.33	2.81	13.85	30.8	355.1
	n	11	11	11	11	11	11	11	11	11	11	11	11
Exercise I	M	9[illegible].5	929.3	80.8	11.79	121.3	—	—	22.2	11.1	119.0	69	823
	SE	3.33	22.60	4.71	0.561	4.42	—	—	1.44	1.42	6.95	6.9	58.6
	SD	11.06	74.95	15.63	1.861	14.65	—	—	4.77	4.48	19.66	21.8	165.8
	n	11	11	11	11	11	—	—	11	10	8	10	8
Exercise II	M	112.5	1325.9	88.4	15.18	135.5	38.1	4.4	21.5	10.0	118.6	57	629
	SE	3.95	50.43	4.88	0.460	3.44	1.68	0.78	1.15	0.91	5.90	7.1	34.8
	SD	12.47	159.45	15.42	1.453	10.87	4.76	2.20	3.63	2.74	18.67	21.2	110.1
	n	10	10	10	10	10	8	8	10	9	10	9	10

Table 8 Means, standard errors of the means (S.E.) and standard deviations (S.D.) of hemodynamic data at rest and during exercise for 36 patients with coronary heart disease separated after clinical diagnosis. Abbreviations as in table 5.

	Heart rate, beats/min	$\dot{V}_{O_2}$ ml/min STPD	a-vO diff ml/l	$\dot{Q}$ l/min	Stroke volume, ml/beat	Blood pressures, mm Hg: RV s	RV ed	PA $\bar{P}$	PCV $\bar{P}$	BA $\bar{P}$	Vascular resistance, dyn sec cm: Pulm.	Systemic
St.p.inf.myocard.												
Rest												
M	74.5	259.2	44.1	5.97	80.9	24.9	4.8	14.7	7.9	110.9	89	1561
SE	2.03	7.74	1.56	0.252	3.66	0.96	0.55	0.68	0.48	3.95	8.9	88.5
SD	8.69	32.85	6.61	1.069	15.52	3.97	2.23	2.87	1.97	15.81	36.8	354.0
	18	18	18	18	18	17	17	18	17	16	17	16
Exercise												
M	114.5	867.5	89.4	9.81	87.6	46.5	8.9	30.7	19.0	130.1	96	1089
SE	3.38	43.87	3.76	0.417	4.96	2.45	1.82	2.46	3.01	4.80	8.1	59.5
SD	14.33	186.15	15.96	1.769	21.03	9.80	7.27	10.43	12.04	20.38	32.4	252.4
	18	18	18	18	18	16	16	18	16	18	16	18
Ang. pect.												
Rest												
M	74.7	260.4	43.0	6.19	83.6	22.1	4.2	13.3	7.0	109.4	88	1466
SE	2.27	10.68	1.69	0.336	4.21	0.83	0.46	0.72	0.62	4.10	7.6	80.9
SD	9.63	45.30	7.18	1.426	17.85	3.44	1.88	3.07	2.57	17.39	30.2	343.2
	18	18	18	18	18	17	17	18	17	18	16	18
Exercise												
M	112.4	926.9	86.6	10.81	96.8	48.7	8.0	32.6	21.3	137.8	88	1094
SE	3.39	53.02	3.03	0.370	5.13	3.33	1.06	2.48	2.62	5.79	10.4	79.3
SD	14.37	2.4.94	12.84	2.417	21.76	11.71	3.52	10.51	10.49	22.42	41.5	307.2
	18	18	18	18	18	11	11	18	16	15	16	15

Table 9 Hemodynamic data obtained at rest and during exercise before and one hour after saline injection in 9 male patients with coronary heart disease. Mean difference ($\bar{d}$) standard error of the mean difference ($S.E._d$) standard deviation of the differences ($S.D._d$) probability (P) of the difference, means ($\bar{x}$) and variation coefficient of a single determination (C). Abbreviations as in table 5

	Heart rate, beats/min	V_{O_2} ml/min STPD	a-$\bar{v}O_2$ diff., ml/l	$\dot{Q}$, l/min	Stroke volume, ml/beat	Blood pressures, mm Hg					Vascular resistance, dyn sec cm^{-5}	
						RV		PA	PCV	BA		
						B	ed	$\bar{P}$	$\bar{P}$	$\bar{P}$	Pulm	Systemic
Rest												
n	9	9	9	9	9	8	8	9	6	8	6	8
$\bar{d}$	+3.3	−16.0	+2.9	−0.3	−13.2	−2.4	−0.9	−0.4	+0.8	−7.1	−8.3	+61.3
SE_d	2.22	10.32	2.24	0.341	4.36	0.60	0.48	0.87	1.11	3.71	15.15	52.77
SD_d	6.65	30.96	6.72	1.022	13.08	1.69	1.36	2.60	2.71	10.49	37.10	149.23
P	>0.1	>0.1	>0.2	>0.05	<0.05	<0.01	>0.1	>0.6	>0.5	>0.05	>0.6	>0.2
$\bar{x}$	77.0	267.7	44.4	6.06	79.9	22.2	4.4	14.1	7.4	108.9	76	1488
C	6.1	8.2	10.7	11.9	11.6	5.4	21.8	13.0	25.9	6.8	34.5	7.1
Exercise												
n	9	9	9	9	9	5	4	9	7	7	7	7
$\bar{d}$	−0.2	−55.7	+0.4	−0.71	−5.6	−0.8	±0	−0.8	±0	+3.7	+5.7	+52.9
SE_d	1.89	23.59	2.02	0.374	3.43	0.49	0.41	0.28	1.00	4.05	7.19	54.05
SD_d	5.67	70.76	6.06	1.123	10.90	1.10	0.82	0.83	2.65	10.72	19.02	143.03
P	>0.9	<0.05	>0.8	>0.1	>0.1	>0.1	>0.9	<0.05	>0.9	>0.4	>0.5	>0.3
$\bar{x}$	112.2	930.4	86.6	10.69	96.0	44.0	8.5	28.4	16.4	128.0	80	996
C	3.6	5.4	5.0	7.4	8.0	1.8	6.8	2.1	11.4	5.9	16.8	10.2

Table 10 Hemodynamic data obtained before and one hour after acute intravenous digitalization with 1.2—1.6 mg of lanatoside C in 21 male patients with coronary heart disease. Mean difference ($\bar{d}$), standard error of the difference (S.E.), standard deviation of the differences (S.D.) and probability (P) of the difference. Abbreviations as in table 5

	Heart rate, beats min	V_{O_2} ml min STPD	a—O diff ml l	Q l min	Stroke volume, ml/beat	Blood pressures, mm Hg					Vascular resistance dyn sec cm⁻⁵	
						RV		PA	PCV	DA		
						s	ed	$\bar{P}$	$\bar{P}$	$\bar{P}$	Pulm.	Systemic
Rest	21	1	21	21	21	18	18	21	20	20	19	20
$\bar{d}$	+2.0	+2.7	−2.0	+0.34	+3.4	−2.3	−1.6	−2.6	−3.0	−0.7	−1.6	−91.0
SE_d	1.71	4.25	1.19	0.156	3.06	1.04	0.49	0.71	0.54	2.63	12.15	59.30
SD_d	7.84	19.46	5.44	0.717	14.04	4.43	2.06	3.25	2.43	11.85	52.94	265.17
P	>0.2	>0.5	>0.1	<0.05	>0.2	<0.05	<0.01	<0.01	<0.001	>0.7	>0.8	>0.1
Exercise	21	21	21	21	21	16	16	21	18	18	18	18
$\bar{d}$	−5.8	−19.6	+1.3	−0.41	+1.1	−4.4	−3.8	−6.9	−6.7	−2.4	+3.3	+35.6
SE_d	1.83	13.12	1.45	0.177	1.59	1.52	0.91	1.73	1.66	2.15	10.16	26.21
SD_d	8.36	60.14	6.6	0.815	7.29	6.08	3.62	7.92	7.01	9.13	43.11	111.21
P	<0.01	>0.1	>0.2	<0.05	>0.4	<0.05	<0.001	<0.001	<0.001	>0.2	>0.7	>0.8

Table 9 Hemodynamic data obtained at rest and during exercise before and one hour after saline injection in 9 male patients with coronary heart disease. Mean difference ($\bar{d}$) standard error of the mean difference ($S.E._d$) standard deviation of the differences ($S.D._d$) probability (P) of the difference, means ($\bar{x}$) and variation coefficient of a single determination (C) Abbreviations as in table 5

	Heart rate, beats/min	V_{O_2} ml/min STPD	a vO_2 diff ml/l	Q l/min	Stroke volume, ml/beat	Blood pressures, mm Hg					Vascular resistance dyn sec cm^{-5}	
						RV		PA	PCV	BA		
						S	ed	$\bar{P}$	$\bar{P}$	$\bar{P}$	Pulm.	Systemic
Rest												
n	9	9	9	9	9	8	8	9	6	8	6	8
$\bar{d}$	+3.3	−16.0	+2.8	−0.73	−13.2	−2.4	−0.9	−0.4	+0.8	−7.1	−8.3	+61.3
SE_d	2.22	10.32	2.24	0.341	4.36	0.60	0.48	0.87	1.11	3.71	15.15	52.77
SD_d	6.65	30.96	6.72	1.022	13.08	1.69	1.36	2.60	2.71	10.49	37.10	149.23
P	>0.1	>0.1	>0.2	>0.05	<0.05	<0.01	>0.1	>0.6	>0.5	>0.05	>0.6	>0.2
$\bar{x}$	77.0	267.7	44.4	6.06	79.9	22.2	4.4	14.1	7.4	108.9	76	1488
C	6.1	8.2	10.7	11.9	11.6	5.4	21.8	13.0	25.9	6.8	34.5	7.1
Exercise												
n	9	9	9	9	9	5	4	9	7	7	7	7
$\bar{d}$	−0.2	−55.7	+0.4	−0.71	−5.6	−0.8	±0	−0.8	±0	+3.7	+5.7	+52.9
SE_d	1.89	23.59	2.02	0.374	3.63	0.49	0.41	0.28	1.00	4.05	7.19	54.05
SD_d	5.67	70.76	6.06	1.123	10.90	1.10	0.82	0.83	2.65	10.72	19.02	143.03
P	>0.9	<0.05	>0.8	>0.1	>0.1	>0.1	>0.9	<0.05	>0.9	>0.4	>0.5	>0.3
$\bar{x}$	112.2	930.4	86.6	10.69	96.0	44.0	8.5	28.4	16.4	128.0	80	996
C	3.6	5.4	5.0	7.4	8.0	1.8	6.8	2.1	11.4	5.9	16.8	10.2

Table 10 Hemodynamic data obtained before and one hour after acute intravenous digitalization with 1.2—1.6 mg of lanatoside C in 21 male patients with coronary heart disease. Mean difference ($\bar{d}$), standard error of the difference (S.E.), standard deviation of the differences (S.D.) and probability (P) of the difference. Abbreviations as in table 9

	Heart rate, beats/min	V_{O_2} ml/min STPD	O_2 diff ml/l	Q l/min	Stroke volume, ml/beat	Blood pressures, mm Hg RV s	RV ed	PA $\bar{P}$	PCV $\bar{P}$	BA $\bar{P}$	Vascular resistance dyn sec cm Pulm.	Systemic
Rest												
	21	21	21	21	21	18	18	21	20	20	19	20
$\bar{d}$	+3.0	+2.7	−2.0	+0.34	+3.4	−2.3	−1.6	−2.6	−3.0	−0.7	−1.6	−91.0
SE_d	1.71	4.25	1.19	0.154	3.06	1.04	0.49	0.71	0.54	2.65	12.15	59.30
SD_d	7.84	19.46	5.44	0.717	14.04	4.43	2.06	3.25	2.43	11.85	52.94	265.17
P	>0.2	>0.5	>0.1	<0.05	>0.2	<0.05	<0.01	<0.01	<0.001	>0.7	>0.8	>0.1
Exercise												
	21	21	21	21	21	16	16	21	18	18	18	18
$\bar{d}$	−5.8	−19.6	+1.5	−0.41	+1.1	−4.4	−3.8	−6.9	−6.7	−2.4	+3.3	+35.6
SE_d	1.83	13.12	1.45	0.177	1.59	1.32	0.91	1.73	1.66	2.15	10.16	26.21
SD_d	8.36	60.14	6.67	0.815	7.29	6.08	3.62	7.92	7.04	9.13	43.11	111.21
P	<0.01	>0.1	>0.2	<0.05	>0.4	<0.05	<0.001	<0.001	<0.001	>0.2	>0.7	>0.8

REFERENCES

Agress, C. A., Jacobs, H. I. Glassner, H. F. Lederer M. A., Clark, W. G. Wroblewski, F. Karmen, A., Laude J. S. Serum transaminase levels in experimental myocardial infarction. Circulation *11* 711 1955

Arén O. Lindgren, E., Malmström, G. Till klinnedom om mätfelen vid Liljestrand Lysholm-Nylin Zachrissons metod för hjärtvolymsbestämning. Nordisk Medicin 1946.

Bayliss, R. I. S., Etheridge M. I. Hyman, A. L., Kelly H. G. McMichael, J. Reid, E. A. S. The effect of digoxin on the right ventricular pressure in hypertensive and ischaemic heart failure. Brit. Heart J *12* 317 1950

Berglund, E. The function of the ventricles of the heart. Acta Physiol. Scand. Suppl 119 1955

Biörck, G. *Anoxemia and exercise tests in the* diagnosis of coronary disease. Am. Heart J *32* 689 1946.

Biörck, G. Hanson, A. Glutamic-oxalacetic transaminase in the diagnosis of myocardial infarction. I. Serum transaminase activity in relation to the clinical picture Acta Med Scand. *155* 317 1956.

Biörck, G. Sievers, J. Blomqvist, G. Studies on myocardial infarction in Malmö 1936 to 1954. III Follow-up studies of a hospital material. Acta Med. Scand. *162* 81 1958

Biörck, G. R habilitering a hjärtsjuka. Svenska *Läkartidningen 61 211 1964.*

Blackburn, H., Keys, A. Simonson E. Rautaharju, P. Punsar S. The electrocardiogram in population studies. A classification system. Circulation *21* 1160, 1960

Boerema, L., Blickman, J. R. Reduced intrathoracic circulation as an aid in angiocardiography J. Thorac Surg. *30* 129 1955

Braunwald E., Frahm, C. J. Studies on Starling's law of the heart. IV Observations on the hemodynamic functions of the left atrium in man. Circulation, *4* 633 1961 (A)

Braunwald, E., *Bloodwell, R. D. Goldberg, L. I.* Morrow A. G. Studies on digitalis. IV Observations in man on effects of digitalis preparations on the contractility of the non-failing heart and on total vascular resistance. J. Clin. Invest. *40* 52, 1961 (B)

Broch, O. J. Jr. Den diagnostiske og prognostiske verdi av transaminasebestemmelser Farmakoterapi, hefte *4* 1963

Cournand, A., Ranges, H. A. Catheterization of right auricle in man. Proc. Soc. Exp. Biol. and Med. *46* 452, 1941

Dexter, L., Whittenberger J. L., Haynes, F. W. Goodale, W. T. Gorlin, R., Sawyer, C. G. Effect of exercise on circulatory dynamics of normal individuals. J. Appl. Physiol. *3* 439 1951

Dixon, W. J. *Massey F. J. J.* Introduction to statistical analysis. McGraw Hill Book Company Inc. 1957

Donald, K. W. Bishop I. M., Cumming, G. Wade O. L. The effect of exercise on the cardiac output and circulatory dynamics of normal subjets Clin. Sci. *14* 37 1955

Drabkin, D. L., Schmidt, C. F. Spectrophotometric studies. J. Biol Chem. *157* 69 1945

Dresdale D. T. Yuceoglu, Y. Z., Michtom, R. J. Schultz, M. Lunger M. Effect of lanatoside C on cardiovascular hemodynamics Acute digitalizing doses in subjects with normal hearts and w th heart disease without failure. Am. J. Cardiol. *4* 88, 1959

Edens, E. Die Strophanthinbehandlung der Angina pectoris. Münch. med. Wschr 1934, No. 37

Eliasch, H. The pulmonary circulation at rest and on effort in mitral stenosis. Scandinav J. Clin. Lab. Invest. Suppl. 4, 1952.

Ferrer, M. I., Conroy R. J. Harvey R. M. Some effects of digoxin pon the heart and circulation in man. *Digoxin in combined (left and right)* ventricular failure Circulation, *21* 372, 1960

Ford, A. B. Hellerstein, H. K.. Energy cost of the Master two-step test. J.A.M.A. 164 1868, 1957

Forsberg, S. A. Paulin, S., Varnauskas, E., Werkö, L. Coronary angiography in the diagnosis of coronary heart disease. Acta Med. Scand. 173 269 1963.

Garland, T. Lindgren, G.. Heart volume determination on microfilm as screening test on sick men. III congrès International de radio-photographie médicale 20—23 août 1958 Bulletin final page 654.

Gorlin, R. Brachfeld, N. MacLeod, C., Bopp P. Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease or increased left ventricular work. Circulation 19 705, 1959

Gorlin, R. Drugs and angina pectoris. Am. J. Cardiol. 9 419 196...

Gorlin, R. Krasnow N. Levine H. J. Messer, J. V. Effect of exercise on cardiac performance in human subjects with minimal heart disease. Am. J. Cardiol. 13 293, 1964.

Granath, A. Jonsson, B., Strandell, T. Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta Med. Scand. 176 425 1964 (A)

Granath, A. Strandell, T. Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. Acta Med. Scand. 176 447 1964 (B)

Gray C. R. Hoffman, H. A. Hammond, W. S. Miller K. L. Oseasohn, R. O. Correlation of arteriographic and pathologic findings in the coronary arteries in man. Circulation 26 494, 196..

Hallén, A. Angina pectoris. A clinical study with special reference to surgical treatment. Acta Chir. Scand. Suppl. 323 1964

Harvey R. M. Ferrer M. I. Cathcart, R. T. Richards, D. W. J. Cournand, A. Some effects of digoxin upon the heart and circulation in man. Digoxin in left ventricular failure. Am. J. Med. 4 9 1949

Harvey R. M. Ferrer, M. I. Cathcart, R. T. Alexander J. K. Some effects of digoxin on the heart and circulation in man. Digoxin in enlarged hearts not in clinical congestive failure. Circulation 4: 366, 1951.

Harvey R. M., Smith, W. M., Parker J. O., Ferrer, M. L. The response of the abnormal heart to exercise. Circulation 26. 341 1962.

Hickam, J. B. Cargill, W. H.. Effect of exercise on cardiac output and pulmonary arterial pressure in normal persons and in patients with cardiovascular disease and pulmonary emphysema. J. Clin. Invest. 27 10, 1948.

Holmgren, A., Mattson, K. H.. A new ergometer with constant work load at varying pedalling rate. Scand. J. Clin. Lab. Invest. 6 137 1954.

Holmgren, A., Pernow B. Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison with the Van Slyke method. Scand. J. Clin. Lab. Invest. 11 143, 1959

Holmgren, A., Pernow B. The reproducibility of cardiac output determination by the direct Fick method during muscular work. Scand. J. Clin. Lab. Invest. 12 224, 1960 (A)

Holmgren, A., Jonsson, B., Sjöstrand, T. Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke volume at different work intensities. Acta Physiol. Scand. 49 343 1960 (B)

Holmgren, A., Strandell, T. On the use of chest head leads for recording of electrocardiogram during exercise. Acta Med. Scand. 169 57 1961.

Johnson, J. B. Fairley A., Carter, C.. Effects of sublingual nitroglycerin on pulmonary arterial pressure in patients with left ventricular failure. Ann. Int. Med. 50 34 1959

Jonsell, S. Method for determination of heart size by teleroentgenography (heart volume index) Acta Radiol. 20 325 1939

Kattus A. A., MacAlpin, R., Longmire, W. P. O'Loughlin, B. J. Bishop H. Coronary angiograms and the exercise electrocardiogram in the study of angina pectoris. Am. J. Med. 34 19 1963.

Keith, N. M., Wagener, H. P. Barker N. W. Some different types of essential hypertension. Their course and prognosis. Am. J. Med. Sci. 197 332, 1939

Kinsella D Troup W McGregor M. Studies with a new coronary vasodilator drug Persantin. Am. Heart J 63 146 1962.

Kjellberg S. R., Rudhe U Sjöstrand, T The relation of the cardiac volume to the weight and surface area of the body the blood volume and the physical capacity for work. Acta Radiol 31 113 1949

Kohn, R. R., Rollerson, E. Studies on the mechanism of the age related changes in swelling ability of human myocardium Circulation Research 7 740 1959

Lagerlöf H Werkö L. Studies on the circulation in man. II Normal values for cardiac output and pressure in the right auricle right ventricle and pulmonary artery Acta Physiol Scand 16 75 1948

Lagerlöf H Werkö, L The effect of Lanatoside C on cardiac output and blood pressure in the pulmonary circulation in patients with compensated and decompensated heart disease Acta Cardiol 4 1 1949

Lepeschkin, E. Exercise tests in diagnosis of coronary heart disease Circulation 2 98 1960

Liljestrand, C Lysholm, E. Nylin, G Zachrisson C. G The normal heart volume in man. Am. Heart J 17 406 1939

Lindgren G Odén, S. A. Heart volume determination on microfilms. Acta Radiol 42 374 1954.

Littman, D Dean, D C. Crowley F B J Gibson, I. T Williams, J A. Clinical coronary angiography Am J Cardiol 7 570 1961

Lown, B., Levine S A. Current concepts in digitalis therapy New England J Med 250 771 1954

Lundberg, A Porjé I G Sjoberg, H E Lika medelsprövning id angina pectoris med cykelergometri. Opusc Med 6 163 1961

McMichael J Sharpey-Schafer E. P The action of intravenous digoxin in man. Quart J Med. 13 123 1944

Malmcrona, R Cramér G V muniken, E Hemodynamic data during rest and exercise of patients who have or have not been able to retain their occupation after myocardial infarction. Acta Med. Scand. 174 557 1963

Marchetti, G V Merlo, L., Antognetti, R. M. The effects of nitroglycerin on the coronary blood flow and oxygen consumption of the myocardium in anaesthetized dogs. Am J Cardiol. 13 51 1964

Master A. M., Nuzie S., Brown, R. C., Parker R. C. Jr The electrocardiogram and the "two-step" exercise: A test of cardiac function and coronary insufficiency Am. J Med. Sci. 207 435 1944

Mattingly T W., The postexercise electrocardiogram. Its values in the diagnosis and prognosis of coronary arterial disease Am. J Cardiol. 9 395 1962.

Messer J V Levine, H J Wagman, R. J Gorlin, R.. Effect of exercise on cardiac performance in human subjects with coronary artery disease Circulation 28 404, 1963

Messer J V Sasahara, A. A., Littman D Gorlin, R. Correlation of coronary anatomy and physiology in man. Circulation 24 997 1961.

Müller O Rørvik, K Hemodynamic consequences of coronary heart disease. Brit Heart J 20 302, 1958.

Nahas, G G Spectrophotometric determination of hemoglobin and oxyhemoglobin in whole hemolyzed blood. Science 113 723 1951

Niles, N R Dotter C. T Coronary radiography and endarterectomy Postmortem study of feasibility of surgery Circulation 28 190 1963

Nomenclature and criteria for diagnosis of diseases of the heart and blood vessels. New York Heart Association, Inc 5th edition 1955

Nordenstrom, B Contrast examination of the cardiovascular system during increased intrabronchial pressure Acta Radiol Suppl 200, 1960

Olson R E Introduction to symposium on the physiology of cardiac muscle Am J Med. 30 649 1961

Page E The actions of cardiac glycosides on heart muscle cells Circulation 30 237 1964

Pipper M. P Disappearance of pathological Q waves after cardiac infarction. Brit. Heart J 20 121 1958

Riley R. L. Himmelstein, A Motley H L Weiner H M Cournand, A Studies of the pulmonary circulation at rest and during exercise in normal individuals and in patients with chronic pulmonary disease Am. J Physiol 152 372, 1948

Rohm, E., Burwell, C. S. Hemodynamic aspects of diffuse myocardial fibrosis. Circulation *16* 750, 1956.

Rodman, T., Gorczyca, C. A., Pastor, B. The effect of digitalis on the cardiac output of the normal heart at rest and during exercise. Ann. Int. Med. *55* 620, 1961.

Rodman, T., Pastor, B. H. The hemodynamical effects of digitalis in the normal and diseased heart. Am. Heart J. *65* 564, 1963.

Rose, G. A. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull. Wld. Hlth. Org. *27* 645, 1962.

Ross, J. Jr., Braunwald, E., Waldhausen, J. A. Studies on digitalis. II. Extracardiac effects on venous return and on the capacity of the peripheral vascular bed. J. Clin. Invest. *39* 937, 1960.

Ross, R. S., Schwartz, N., Gaertner, R. A., Friesinger, G. C. Electrocardiographic and hemodynamic observations during selective coronary cineangiocardiography. J. Clin. Invest. *41* 395, 1962.

Russek, H. I. Master two-step test in coronary artery disease. J.A.M.A. *165* 1772, 1957.

Seldinger, S. I. Catheter replacement of the needle in percutaneous arteriography. A new technique. Acta Radiol. *39* 368, 1953.

Selzer, A., Ebnother, C. L., Packard, P., Stone, A. O., Quinn, J. E. Reliability of electrocardiographic diagnosis of left ventricular hypertrophy. Circulation *17* 255, 1958.

Selzer, A., Hultgren, H. N., Ebnother, C. L., Bradley, H. W., Stone, A. O. Effect of digoxin on the circulation in normal man. Brit. Heart J. 1 5 1959.

Selzer, A., McCaughey, D. J. Hemodynamic patterns in chronic cardiac failure. Am. J. Med. *28* 37, 1960.

Selzer, A., Malmborg, R. O. Hemodynamic effects of digoxin in latent cardiac failure. Circulation *25* 695, 1962.

Simonson, E., Keys, A. The electrocardiographic exercise test. Changes in the scalar ECG and in the mean spatial QRS and T vectors in two types of exercise: effect of absolute and relative body weight and comment on normal standards. Am. Heart J. 85 956

Sjöstrand, T. Changes in the respiratory organs of workmen at an ore smelting works. Acta Med. Scand. Suppl. *196* 687, 1947.

Sjöstrand, T. A method for the determination of the total hemoglobin content of the body. Acta Physiol. Scand. *16* 211, 1948.

Sjöstrand, T. The electrocardiographic work and hypoxemia tests. Scand. J. Clin. Lab. Invest. *3* 1 1951.

Sjöstrand, T. Functional capacity and exercise tolerance in patients with impaired cardiovascular function. Clinical cardiopulmonary physiology. Grune & Stratton, Inc., New York 1960.

Sloman, G., Hare, W. S. C. Clinical application of coronary angiography. Med. J. Australia *47* 611, 1960.

Snedecor, G. W., Cochran, W. G. Statistical methods applied to experiments in agriculture and biology. 5th edition. Ames, Iowa State College Press 1957.

Sokolow, M., Lyon, T. P. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am. Heart J. *37* 161, 1949.

Solem, J. H., Helle, I., Jörgensen, W. A follow-up study of 330 patients with myocardial infarction, with particular reference to development of heart failure. Acta Med. Scand. *174* 515, 1963.

Stokes, J. III, Dawber, T. R. The "silent coronary". The frequency and clinical characteristics of unrecognized myocardial infarction in the Framingham study. Ann. Int. Med. *50* 1359, 1959.

Strandell, T. Circulatory studies on healthy old men. Acta Med. Scand. Suppl. 414, 1964 (A).

Strandell, T. Heart rate, arterial lactate concentration and oxygen uptake during exercise in old men compared with young men. Acta Physiol. Scand. 60 197, 1964 (B).

Strandell, T. Heart volume and its relation to some anthropometric data in old men compared with young men. Acta Med. Scand. 176 205, 1964 (C).

Strandell, T. Total hemoglobin, blood volume and hemoglobin concentration at rest and circulatory adaption during exercise in relation to some anthropometric data in old men compared with young men. Acta Med. Scand. 176 19, 1964 (D).

Kinsella, D Troup W., McGregor M., Studies with a new coronary vasodilator drug: Persantin. Am. Heart J 63 146, 1962.

Kjellberg, S. R., Rudhe U Sjöstrand, T., The relation of the cardiac volume to the weight and surface area of the body the blood volume and the physical capacity for work. Acta Radiol. *31* 113 1949

Kohn, R. R., Rollerson, E. Studies on the mechanism of the age-related changes in swelling ability of human myocardium. Circulation Research 7 740 1959

Lagerlöf H Werkö L. Studies in the circulation in man. II. Normal values for cardiac output and pressure in the right auricle right ventricle and pulmonary artery Acta Physiol. *Scand 16 75 1948.*

Lagerlöf H Werkö L. The effect of lanatoside C on cardiac output and blood pressure in the pulmonary circulation in patients with compensated and decompensated heart disease. Acta Cardiol *4* 1 1949

Lepeschkin, E. Exercise tests in diagnosis of coronary heart disease Circulation *2* 986, 1960

Liljestrand G Lysholm, E., Nylin, G Zachrisson, C. G., *The normal heart vol me in man.* Am. Heart J *17* 406, 1939

Lindgren, G Odén, S A Heart volume determination on microfilms. Acta Radiol *42* 374, 1954.

Littman, D Dean D C., Crowley F B. J Gilson, L T W lliams, J A Cl ical coronary angiography Am J Cardiol *7 570 1961*

Lown, B., Levine S. A Current concepts i digital s therapy New England J Med. *250* 771 1954.

Lundberg, A., Porjé I G Sjoberg, H E. Lakemedelsprövning vid angina pectoris med cykel ergometri Opusc. Med. 6 163 1961

McMichael J Sharpey Schafer E. P The action of ntravenous digoxin in man. Quart J Med. *13* 123 1944.

Malmcrona, R., *Cramér* G Varnauskas, E. Hemodynamic data during rest and exercise for patients who have or *have* not *been able to* retain their occupation after myocardial infarction. Acta Med. Scand. *174* 557 1963.

Marchetti, G V Merlo, L., Antonetti, R. M The effects of nitroglycerin on the coronary blood flow and oxygen consumption of the *myocardium in anesthetized* dogs. Am. J Cardiol *13* 51 1964.

Master A. M., Nuzie, S Brown, R. C., Parker R. C. Jr The electrocardiogram and the "two-step" exercise: A test of cardiac function and *coronary insufficiency* Am. J Med. Sci. *207* 435 1944.

Mattingly T W., The postexercise electrocardiogram. Its values in the diagnosis and prognosis of coronary arterial disease Am. J Cardiol *9 395 1962.*

Messer J V Levine H J Wagman, R. J Gorlin, R., Effect of exercise on cardiac performance in human subjects with coronary artery disease. Circulation *28* 404, 1963

Messer J V Sasahara, A. A., Littman, D Gorlin, R. Correlation of coronary anatomy and physiology in man. Circulation *24* 997 1961

Müller O Rorvik, K. Hemodynamic consequences of coronary heart disease Brit. Heart J *20 302, 1958.*

Nahas, G G Spectrophotometric determination of hemoglobin and oxyhemoglobin in whole *hemolyzed blood. Science 11* 723 1951

Niles, N R. Dotter C. T Coronary radiography and endarterectomy Postmortem study of feasibil ty of surgery Circulation *28* 190 1963

Nomenclature and criteri for diagnosis of diseases *of the heart and blood* essels. New York Heart Association, Inc. 5th edition 1955

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Pappas, M P Disappearance of pathological Q-waves after cardiac nfarction. *Brit. Heart J* 20 123 1958.

Riley R. L Himmelstein, A., Motley H. L *Weiner, H M., Cournand, A.* Studies of the pulmonary circulation at rest and during exercise in normal individuals and *in patients with* hronic pulmonary disease Am. J Physiol. *152* 372, 1948

Robin, E., Burwell, C. S. Hemodynamic aspects of diffuse myocardial fibrosis, Circulation 16 750, 1956.

Rodman, T. Gorczyca, C. A. Pastor, B. The effect of digitalis on the cardiac output of the normal heart at rest and during exercise. Ann. Int. Med. 55 620, 1961

Rodman, T. Pastor, B. H. The hemodynamical effects of digitalis in the normal and diseased heart. Am. Heart J. 65 564, 1963.

Rose, G. A. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull. Wld. Hlth. Org. 27 645 1962.

Ross, J. J. Braunwald, E., Waldhausen, J. A. Studies on digitalis. II. Extracardiac effects on venous return and on the capacity of the peripheral vascular bed. J. Clin. Invest. 39 937 1960

Ross, R. S. Schwartz, N., Gaertner, R. A., Friesinger, G. C. Electrocardiographic and hemodynamic observations during selective coronary cineangiocardiography. J. Clin. Invest. 41 593, 196.

Russek, H. I. Master two-step test in coronary artery disease. J.A.M.A. 165 1772, 1957

Seldinger, S. I. Catheter replacement of the needle in percutaneous arteriography. A new technique. Acta Radiol. 39 368, 1953

Selzer, A. Ebnother, C. L. Packard, P. Stone, A. O. Quinn, J. E. Reliability of electrocardiographic diagnosis of left ventricular hypertrophy. Circulation 1 255 1958

Selzer, A. Hultgren, H. N. Ebnother, C. L. Bradley, H. W. Stone, A. O. Effect of digoxin on the circulation in normal man. Brit. Heart J. 1959

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Selzer, A. Malmborg, R. O. Hemodynamic effects of digoxin in latent cardiac failure. Circulation 5 695 1962

Simonson, E. Keys, A. The electrocardiographic exercise test. Changes in the scalar ECG and in the mean spatial QRS and T vectors in two types of exercise: effect of absolute and relative body weight and comment on normal standards. Am. Heart J. 5 1956

Sjöstrand, T. Changes in the respiratory organs of workmen at an ore smelting works. Acta Med. Scand. Suppl. 196 687 1947

Sjöstrand, T. A method for the determination of the total hemoglobin content of the body. Acta Physiol. Scand. 16 211, 1948.

Sjöstrand, T. The electrocardiographic work and hypoxemia tests. Scand. J. Clin. Lab. Invest. 3 1 1951

Sjöstrand, T. Functional capacity and exercise tolerance in patients with impaired cardiovascular function. Clinical cardiopulmonary physiology. Grune & Stratton, Inc., New York 1960

Sloman, G. Hare, W. S. C. Clinical application of coronary angiography. Med. J. Australia 47 611 1960.

Snedecor, G. W. Cochran, W. G. Statistical methods applied to experiments in agriculture and biology. 5th edition. Ames, Iowa State College Press. 1957

Sokolow, M., Lyon, T. P. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am. Heart J. 37 161 1949

Solem, J. H. Hjelle, L., Jörgensen, W. A follow-up study of 330 patients with myocardial infarction, with particular reference to development of heart failure. Acta Med. Scand. 174 315 1963

Stokes, J. III, Dawber, T. R. The "silent coronary". The frequency and clinical characteristics of unrecognized myocardial infarction in the Framingham study. Ann. Int. Med. 50, 1359 1959

Strandell, T. Circulatory studies on healthy old men. Acta Med. Scand. Suppl. 414, 1964 (A)

Strandell, T. Heart rate, arterial lactate concentration and oxygen uptake during exercise in old men compared with young men. Acta Physiol. Scand. 60 197 1964 (B)

Strandell, T. Heart volume and its relation to some anthropometric data in old men compared with young men. Acta Med. Scand. 176 205, 1964 (C)

Strandell, T. Total hemoglobin, blood volume and hemoglobin concentration at rest and circulatory adaption during exercise in relation to some anthropometric data in old men compared with young men. Acta Med. Scand. 176 219 1964 (D)

Strandell T. Heart rate and work load at maximal working intensity in old men. Acta Med. Scand. *176* 301 1964 (E)

Söderholm, B., Thulesius, O Heyman, F Malmcrona, R., Björntorp P Myocardial infarction in the younger age groups. III Follow up observations with special reference to exercise tolerance tests Acta Med. Scand. *172* 585 1962.

Varnauskas, E Studies in hypertensive cardiovascular disease with special reference to cardiac function. Scand. J Clin. Lab. Invest. *7* Suppl. 17 1955

Wahlberg, F A study of acute myocardial infarction at the Seraphimer hospital during 1950—1959 Am. Heart J *65* 749 1963

Wahlund H. *Determination of the physical* working capacity A physiological and clinical study with special reference to standardization of cardio-pulmonary functional tests. Acta Med. Scand Suppl 215 1948

Werkö L., Lagerlöf H. The pulmonary capillary venous pressure in man. Resumés Prem. Congr Mond. Card. Baillière, Paris 144, 1950.

Report of a WHO expert committee: Rehabilitation of patients with cardiovascular disease. Wld Hlth Org techn. Rep Ser 70, 1964.

Widimsky J Berglund, E., Malmberg, R. Effect of repeated exercise on the lesser circulation. J Appl Physiol. *18* 983 1963

Wiggers, C. J Circulatory dynamics. Physiologic studies. Grune & Stratton, New York 1952.

Wiklander, O Blood volume determinations in surgical practice Acta Chir. Scand. Suppl. 208, 1956.

Williams, M. H., Zohman, L. R., Ratner, A. C. Hemodynamic effects of cardiac glycosides on *normal subjects during rest and exercise. J* Appl Physiol. *13* 417 1958.

Åstrand, P O Experimental studies of physical working capacity in relation to sex and age. Munksgaard, Copenhagen 1952.

Strandell T Heart rate and work load at maximal working intensity in old men. Acta Med Scand. *176* 301 1964 (E)

Söderholm, B., Thulesius, O Heyman, F Malmcrona, R., Björntorp, P.. Myocardial infarction in the younger age groups *III* Follow up observations with special reference to exercise tolerance tests. Acta Med. Scand *17* 585 1962.

Varnauskas, E. Studies in hypertensive cardiovascular disease with special reference to cardiac function. Scand. J Clin. Lab. Invest. *7* Suppl 17 1955

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Werkö L., Lagerlöf H The pulmonary capillary venous pressure in man. Resumés Prem. Congr. Mond. Card. Baillière, Paris 144, 1950.

Report of a WHO expert committee Rehabilitation of patients with cardiovascular disease. Wld Hlth Org. techn. Rep Ser., 270, 1964.

Widimsky J Berglund, E Malmberg, R. Effect of repeated exercise on the lesser circulation. J Appl Physiol. *18* 983 1963

Wiggers, C. J Circulatory dynamics. Physiologic studies. Grune & Stratton, New York 1952.

Wiklander, O Blood volume determinations in surgical practice Acta Chir Scand. Suppl. 208, 1956.

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 4[illegible]5

PROCEEDINGS OF T[illegible]

THIRD INTERNATIONAL CONFERENCE ON SARCOIDOSIS

STOCKHOLM SEPTEMBER 11—14 1963

Editor
SVEN LÖFGREN

Assistant Editors
Hanns J Bauer and Barbro Wecht

ACCOMPANIES VOL. 176

STOCKHOLM 1964

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As an indication of the interest taken in the disease by lung physicians and other interests — pathologists, biochemists, bacteriologists, and virologists — an international symposium was arranged in London in 1958. The initiative was taken by Dr D. Geraint James and the symposium was arranged in collaboration with Professor John G. Scadding. The transactions were not published, but valuable personal contacts were established. Thanks to Professor Martin M. Cummings a second similar conference was held in Washington in 1960. The proceedings of the Washington Conference were published as a supplement to the American Review of Respiratory Diseases (vol. 84, No. 5, part 2 of two parts, 1961).

In September 1963 a third international conference on sarcoidosis was held in Stockholm. The proceedings are presented here. The international basis of this conference was somewhat broader than before: [illegible] six countries were represented. It might be asserted that no new developments resulted from this conference as far as etiological aspects are concerned. On the other hand, the world wide epidemiological survey and the pooling of experience from many fields of research afforded new ideas and created new inspiration for further and even more work on the fascinating problem of sarcoidosis.

Sven Löfgren

Foreword

So far three main stages of development can be distinguished in the history of the disease or syndrome which w now call sarcoidosis.

As is well known, it was originally recog nized as skin complaint by Hutchinson in 1869 as "Mortimer' malady" by Besnier in 1889 as Lupus pernio and by Boeck in 1899 as Multiple benign sarcoid"

The second stage was reached when Schaumann in his study "Sur le lupus pernio 1914 pointed out first that lupus pernio and Boeck sarcoid were variations of one and the same disease and, moreover that these complaints were only occasional skin manifestations of an internal disease which might affect many organs of the body lymph nodes, tonsils, bone marrow spleen, liver and lungs. He suggested new name for the disease "Lymphogranulomatosis benigna

After Schaumann had made his impor tan contribution to the study of the disease more and more cases of generalized lymphogranulomatosis benigna were reported in the literature Heerfordt's Febris uveoparotidea (1909) and Jungling "Ostitis tuberculosa multiplex cystica (1919—21) ere included as manifestations of lymphogranulomatosis benigna. Affections of the heart, skeletal muscles, central nervous system, and kidneys — sometimes combined with hypercalcemia — were recognized also as manifestations of the disease.

However in spite of this, during the three decades following the publication of Schaumann work in 1914 the disease was mainly of interest to dermatologists. As evidence of this interest it may be mentioned that the session of the Réunion Dermatologique in 1934 was devoted to discussion of Boeck oid.

In 1937 Schaumann summarized his ex *perience of the etiology* clinical feature, and prognosis of the disease as follows "There are many observations indicating tuber culous etiology — The most usual course is, that classical tuberculosis manifests itself in the lungs, peritoneum, etc., causing death. Another possible development is, that the disease continues to be benign and, for several years, is comparatively asymptomatic. The process progresses, however especially in the hematopoetic system, until ultimately death follows owing to debility usually combined with severe dyspnea and cardiac weakness. Thus, the prognosis is by no means good, and the benignity indicated in the name should be regarded as only relativ and referring to the protracted course of the disease and, for long time its insignificant effect on the general condition. (Nord. Med. Tidskr *13*, 961 1937)

In the middle of the forties the scope of the disease was widened through third stage of development. The early stage of lymphogranulomatosis benigna was then recognized It was manifested by bilateral hilar lymphadenopathy of the lungs, often combined with erythema nodosum. Through mass chest radiography many cases of early asymptomatic sarcoidosis were also detected. Contrary to the former view that lymphogranulomatosis benigna was rare disease with bad prognosis, it was now evident according to reports from many regions, that it was a rather common disease and in its early stage had de cidedly good prognosis. Owing to the obser vations mentioned, interest was focused especially on pulmonary localization. Sarcoidosis was accepted internationally as the new name of the disease

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Opening of Conference

ARTHUR ENGEL

M. D. Director General
Swedish National Board of Health

When the chairman of the organizing committee, Dr Löfgren, kindly asked me to open the Third International Conference on Sarcoidosis he had no difficulties in persuading me to do so. As an old clinician of internal medicine I was witness of the long and fascinating history of the establishment of sarcoidosis as clinical entity and deeply interested in its puzzling and multifaceted diagnosis. In my present position as public health administrator I feel myself very much engaged in the epidemiological aspects — rather neglected field.

I Sweden, as in most other countries, general morbidity statistics are poor

T fill my own lack of knowledge of the frequency of sarcoidosis I asked my bureau of statistics to pick out from the annual reports of all hospital departments of internal medicine pediatrics and chest clinics inclusive sanatoriums, all cases of sarcoidosis reported in the last year (1962) 389 men and 527 women were found, in total 916 cases, and corresponding to 0.13 /₀₀ of the population. Very few cases were reported from child departments.

The results of the follow-up of the periodical chest mass radiography of the Swedish population are also of greatest interest in this context. I will demonstrate slide which Prof. Wegelius, the head of our mass radiography unit kindly produced on my request. It illustrates the number of observations of sarcoidosis and tuberculosis according to age and sex. The final diagnosis as established through complete clinical examination has been used. The observations were made during 9 year period. The relative prevalence

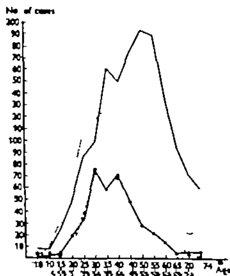


Fig. 1 Active tuberculosis and sarcoidosis detected through mass radiography in Sweden 1953—1961 (Center of Mass Radiography of The Royal Medical Board, Sundbyberg, Sweden. Dr S. Wiljström.)

Active Tuberculosis		Sarcoidosis	
1,504 males	———	396 males	•— —•
867 females		471 females	

of sarcoidosis according to this method of registration is 0.38 ‰ — a figure thrice that of hospitalized cases I just mentioned.

Sarcoidosis, no doubt, is a disease of such importance that it is a fully justified public health measure to introduce a compulsory notification, at least for a specific period, for the purpose of clarifying its prevalence and distribution in the population and with the ultimate perspective of contributing to the search on its etiology. For the time being a special commission has been set up in this country to review the legal background for our fight against all communicable diseases. Sarcoidosis will come up in this connection.

The two upper curves represent the number of cases of TB of the lungs. Kindly contemplate the relatively high figures for TB among old men. The next step of our TB eradication programme will be to intensify the search for cases in these age groups of men. They seem to be found mainly among inebriates, tramps and other asocial individuals, and very often being contagious, they represent a dangerous source of infection.

I strongly feel that your third international conference on sarcoidosis will be successful. Mutual international efforts to attack the many unsolved problems of the disease to which you are devoting your studies are highly indicated and a conference with a limited number of participants of the most qualified capacity is, no doubt, the most effective instrument to serve this purpose.

On behalf of the Swedish health authorities and of the main sponsor of your conference, the Swedish National Association against Heart and Chest diseases, I greet you very welcome to this country where I do hope you will spend a pleasant and from the scientific point of view also fruitful time. Finally I have the pleasure to accomplish the honorable task you offered me. I declare this Third International Conference on Sarcoidosis opened.

From St. Göran Hospital, Stockholm, Sweden.

Concepts of Sarcoidosis

Sven Löfgren

In an introductory speech at this conference I will try to give brief outline of the different and widely divergent views on the concept, or definition, of sarcoidosis.

Originally sarcoidosis (or Besnier-Boeck Schaumann disease, or lymphogranulomatosis benigna) was regarded as special form of tuberculosis. The strange facts, that tubercle bacteria were seldom found in these cases and that, as rule, the sensitivity to tuberculin was either reduced or entirely absent, were explained by presuming either that the reactivity of the host was changed, or that the property of the infecting organism, the tubercle bacterium, was altered.

Thirty years ago, the theory of sarcoidosis as tuberculous disease was generally accepted in many parts of the world. Nowadays, with few exceptions, it has been abandoned by most authors.

According to another concept, sarcoidosis is non-specific syndrome, defined by the presence of the sarcoid tissue reaction. As this reaction will be discussed in the following session it is not necessary to describe it now It will suffice to emphasize that, from an etiological point of view the reaction is nondescript and, accordingly can be provoked by number of agents bacteria and fungi of different kinds, foreign bodies, tumours, etc.

The strict histopathological definition of sarcoidosis was formulated by Scadding (9) I will try to illustrate this concept by diagram (fig. 1)

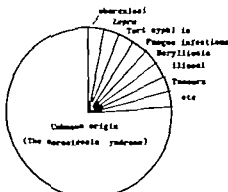


Fig. 1 The sarcoid tissue reaction in conditions of known and unknown origin.

The total area of the circle represents all cases with an established sarcoid tissue reaction. It is proviso that characteristic sarcoid lesions should be present in all tissues affected. If, in such patient, tubercle bacteria are found, Scadding classifies the case as "tuberculous sarcoidosis"; if the reaction occurs in patient with, for example, berylliosis, it is then case of "beryllium sarcoidosis"; if the reaction takes place in patient where no etiological factor can be demonstrated, it is termed "sarcoidosis of unknown origin" To the extent that possibly more and more etiological factors will be demonstrated in the future, the last-mentioned group — sarcoi-

doms of unknown origin — will apparently decrease

One question is, how big are the different etiologic sectors, and, especially how should the size of the tuberculous sector be estimated? In a material consisting of 250 sarcoidosis patients from Brompton Hospital Scadding (10) has reported 18 cases (8 %) in which tubercle bacteria were found in the sarcoid phase, without any change being observed in the clinical picture, which remained that associated with sarcoidosis. Nor was there any significant response to antituberculous drugs in these cases. In five other cases (2 %) tubercle bacteria were found during transition from the clinical picture of sarcoidosis to that of caseating tuberculosis. As far as I can see these figures are taken as a proof, or an indication of an immune-biological transition between sarcoidosis and tuberculosis, in one or th other direction Another explanation of this relationship seems more likely but for comparison I will mention the corresponding figures btained from a material of 700 sarcoidosis patients t St Göran s hospital 1 7 and 0.6 per cent respectively These differences are interesting nd I think they can be explained by the different way in which the Brompton and the St. Göran materials were selected. I will deal with this question in another paper (8)

According to a third concept the definition is based on clinical criteria. I think that nowadays most of the workers in the field of sarcoidosis follow this line. This concept, too, postulates

1 that the sarcoid tissue reaction is non descript in nature,

2. that it should be present n all cases classified as sarcoidosis and in all organs affected.

Now I want to return to the diagram. According to the concept in question, the cases belonging to the groups with a known etiology — tuberculosis, lepra, syphilis, berylliosis, moniliasis and so on — represent a definite minority among the cases with a sarcoid tissue reaction. Furthermore, within this minority the clinical picture varies greatly: a case of tuberculosis is not similar to a case of berylliosis, etc. On the other hand, when we consider the cases of unknown etiology (or etiologies) this group constitutes the majority And this large group presents so many clinical features common to all the cases, that we feel inclined to speak of a clinical entity

The difference between the histopathological and the clinical definitions is obvious and it is necessary to make this difference clear On the other hand, once these different standpoints are defined, we shall have every chance of a fruitful discussion. And, to summarize we can with the help of the diagram visualize, in a very simple way the difference between the two schools The term, or concept of sarcoidosis is, for the supporters of the histopathological definition, represented by the total area of the circle whereas, for the adherents of the clinical definition, it is represented by the sector w th unknown etiology

According to the clinical definition of sarcoidosis, the disease, or syndrome, is etiologically independent of tuberculosis. The reasons for this assumption may be reviewed briefly

1 The clinical picture of sarcoidosis is not very similar to that of classical tuberculosis. Among other things, different organs are affected in the two conditions.

2. The histopathological tissue changes in the two conditions have no greater reciprocal resemblance than those found in all the diseases enumerated previously which can give rise to sarcoid reaction.

3 The relative or the absolute tuberculin anergy which, in the form of positive anergy is regarded by the adherents of the tuberculous theory as supporting this conception, can more simply be considered as an argument *against* th ew that sarcoidosis is a tuberculous complaint. In this connection the occurrence of erythema nodosum in sarcoidosis is a noteworthy phenomenon In principle, erythema nodosum may be interpreted as a manifestation of hyperergia. If sarco dosis were caused by tuberculous infection how can it be explained that, simultaneously with *tuberculin anergy* it reacts with the *hyperergic* symptom, erythema nodosum (1)

4 The two conditions have an essentially different ge distribution, since with few exceptions, sarcoidosis first occurs at the age of sexual maturity

5 In many parts of th world tuberculosis

is rapidly declining. At the same time in the same regions the frequency of sarcoidosis either remains unchanged or is possibly even increasing.

6. The antibacterial drugs for tuberculosis have no effect on sarcoidosis. On the other hand, corticosteroids, which, without the protection of chemotherapeutic agents, are contraindicated in tuberculosis, can be used successfully in sarcoidosis.

7 Finally social aspect may be mentioned which is not devoid of interest. It is matter of general experience that tuberculosis patients belong, to a relatively large extent, to problematical social group, consisting of the clientele of hostels, alcohol addicts and vagabonds. Experience gained from large material has taught us that it is extremely rare that sarcoidosis patients are encountered in these groups (7) W have attempted to explain this difference by assuming that asociality in its various forms, with its resulting undernourishment, constitutes an important factor in the pathogenesis of tuberculosis. T all appearances this factor is of minor importance in the development of *sarcoidosis* whereas in this disease hormonal factors play significant pathogenetic role.

One thing that ought to be done now is to describe the clinical criteria upon which the third concept of sarcoidosis is founded. For two reasons I will refrain from such a description First, it may be unnecessary for this audience of sarcoidosis specialists. Second, the time for all speakers is restricted.

I wish only to mention few principles. As in tuberculosis, so in sarcoidosis it is essential to distinguish between the early and the chronic stage (4 6) Regarding sarcoidosis, the early stage no doubt presents the most characteristic picture The bilateral hilar lymphoma syndrome with or without erythema nodosum, with typical joint symptoms, in females often related to the period of lactation, with, as rule decidedly good prognosis — this syndrome can scarcely be confused with any other disease (1 2)

Admittedly the generalized or chronic stage sometimes offers a more confusing picture. The pulmonary lesions with patchy densities, fibrosis, and emphysema may be nondescript and have similarities to other chronic pulmonary diseases. In these cases, however w often have recourse to the extrapulmonary changes which are frequently present. Some of them are nearly pathognomonic in character Here I have in mind uveoparotitis, typical sarcoids of mucosae and skin — in particular scar sarcoids (3) — and osseous metastases.

Among abnormal findings in the field of blood chemistry w has — in the active, chronic stage — hyperglobulinemia, mainly of the gamma fraction, but also of the alpha, and beta-fractions. Occasionally hypercalcemia is found; this phenomenon is usually connected with peculiar disturbance of the kidney function (5)

The clinical concept does not give a precise or final definition of sarcoidosis, but it provides useful frame of reference for practical clinical work.

None of the concepts described solves the riddle of sarcoidosis as the etiology and pathogenesis still remain obscure. It seems that sarcoidosis with its many strange features belongs to borderland with similarities to established infections and still etiologically obscure conditions as collagenoses, Hodgkin disease and real tumor diseases.

References

1 Löfgren, S. Acta med. Scand. Suppl. *124* 174 1946.
2. Löfgren, S. Ibid. *145* 424 465, 1953.
3. Löfgren, S Snellman, B. and Nordenstam, H. Acta chir Scand. *108*. 405, 1955.
4 Löfgren S. Trans. 4th Commonw Health & Tuberc. Conf. 1955 78.
5. Löfgren, S., Snellman, B. and Lindgren, A. G. H. Acta med. Scand. *159* 295 1957
6. Löfgren, S. Acta tub. Scand. Suppl. *45*. 13, 1959
7 Löfgren S. Ibid. Suppl. *45*: 19 1959
8. Löfgren, S. Proc. 3rd Intern. Conf. on Sarcoidosis Acta med. Scand. Suppl. *425*: 268, 1964.
9 Scadding, J G. Brit. med. J 1950 745.
10. Scadding, J G. Ibid. 1960 1617

Dr SCADDING I cannot hope n the short time available to deal with all the points of difference between Dr Löfgren s views and mine. Some of our differences may be traceable to different *definitions of the general concept a disease"* a matter which I have discussed elsewhere (Scadding 1959 1963) I define a disease" as the sum of those abnormal phenomena which are bserved in a group of living organisms with disturbed structure or function, the group being defined in a stated way The group from the characteristics of which the concept of an individual disease is an abstraction may be defined in several different ways in simple descriptive terms, in terms of morbid anatomy in term of disordered function, or in terms of specific causation. I define sarcoidosis in terms of morbid histology but specify that th specific histological changes must be present in a number of ffected organs. This simple addition invalidates the objection frequently raised to a histological definition of sarcoidosis, that it does not distinguish between local sarcoid reactions and the systemic disease sarcoidosis. In just the same way the systemic disease polyarteritis nodosa is defined as a disease characterised by the presence of the specific histological changes in vessels in number of affected organs and tissues and no confusion is caused by the occasional finding of isolated changes of the same histological pattern in cases which on other grounds must be placed n som other nosological group. I thus *have no difficulty in excluding from the category* sarcoidosis all those sectors in Dr Löfgren dia gram relating to such things as foreign bodies and malignant tumours. I am afraid he has also over simplified my views on the possible role of mycobacterial infection. I will not anticipat what I have to say on this subject later in the week but *I would like to comment on the figure 8 % which* his diagrams suggests that I ttribute to *M tuberculosis*. This is in fact the percentage of the 230 cases, analysed in 1960 in which tubercle bacilli had been found at a time when the disease was in *other respects characteristic of sarcoidosis 5 %* more had had various forms of caseating tuberculosis in the past, some of them in close temporal relation to the development of features of sarcoidosis and 2 % changed to a frankly caseating phase with demonstrable tubercle bacilli lat in the course of sarcoidosis. Thus there were more than 8 % in which *M tuberculosis* had been found t a time which suggested that t might have been concerned in the causation of the sarcoidosis and I advanced general considerations which led me to the tentative opinion that most of the cases I saw in England were of the same aetiological group But I emphasised two points first, the extreme importance of th immunological reactivity of the host and second, that the possibility that other agents might precipitate sarcoidosis in a susceptible individual must be left open. Thus, the 8 which Dr Löfgren quoted misrepresents my views in several ways. It oversimplifies my concept of the way in which mycobacterial and possibly som other infections may be concerned in the pathogenesis of sarcoidosis t suggests numerical precision which I did not ttempt and paradoxically t th same time understates my tentati e estimate, based on qualitative considerations, of the true importance of mycobacterial *infection in th aetiology of sarcoidosis in England*

References

1 SCADDING, J G Lancet *1* 323 1959
2 SCADDING, J G Brit. med J *2* 1617 1960.
3. SCADDING, J G Brit. med. J *2* 1425, 1963.

PATHOLOGICAL ASPECTS OF SARCOIDOSIS

Moderator Martin M. Cummings

Histopathology — Histochemistry

From the Institute of Pathology University of Zürich, Switzerland.

The Sarcoid Tissue Reaction

The Origin and Significance of Inclusion Bodies. Differential Diagnosis with Particular Delineation from Tuberculosis.

E. Uehlinger

The conference on sarcoidosis, which met in Washington, D. C., in 1948, defined sarcoidosis as follows: "Sarcoidosis is a disease of unknown etiology characterized pathologically by epitheloid tubercle with inconspicuous or no necrosis, occurring in any organ or tissue and by the frequent presence of refractile or apparently calcified bodies in the giant cells of the tubercles." It would be difficult to improve upon this definition even in the light of modern medicine and research, for the basic etiological question remains as yet unclarified. However the interplay and actual significance of various immunological processes in the pathogenesis of the disease has been definitively demonstrated by Löfgren, Kveim, Refvem and Siltzbach.

Löfgren (7) described the bilateral hilus syndrome appearing most significantly with erythema nodosum, and also with other allergic disorders, as an early manifestation of sarcoidosis, a view which is today generally accepted.

The finest formulation of the dynamic disease processes as contained within the concept of erythema nodosum was presented by Wallgren, who basically regarded erythema nodosum not as tuberculous metastasis, but rather as the equivalent of an exanthematous component of an acute infectious disease. It thus represents essentially the full sensitization of the individual and the primary visual allergic expression of the disease progression after the incubation period. Therefore, the disease itself is an external display of an antigen-antibody reaction.

A close correlation has also been observed between the prevalence of erythema nodosum and positive responses to the Kveim antigen during active sarcoidosis. The Kveim reaction has become of great diagnostic value because of its high degree of specificity as developed by Siltzbach. The positive reaction to an intracutaneous injection of antigen is characterized by the proliferation of noncaseating epithelioid cell granulomas which, in a figurative sense, is the morphological equivalent to this localized antigen-antibody reaction.

Refvem, after many finely formulated and



Fig 1 Sarcoid granuloma hyalinization A. Helene 40 years. SN 260/62 (Gieson stain, x 135)

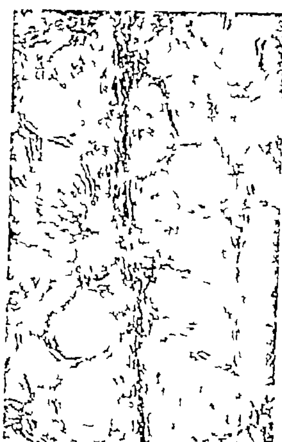


Fig 2 Sarcoid gran loma in hyalinization. Final stage. Preserving pattern. Man, 60 years. SN 838/60 (Path. Inst. München) (v Gieson stain, 46)

carefully executed experiments, showed conclusively that sarcoid antigen and Mycobacterium tuberculosis were not the only substances able to elicit a sarcoid tissue reaction. Other bacteria, viruses, fungi protozoa, and certain organic and inorganic substances also proved capable of inducing the same im munological response

Refvem further asserts that histiocytes are transformed into epithelioid cells through the focal accumulation of exogenous and endogenous phosphatides. This theory is suppor ted by

1 The demonstration of phosphatides in sarcoid and quarz granulomas.

2 The experimental production of epithelioid cell granulomas after intracutaneous injection of phosphatid extracts from human serum or egg white.

3 The production of epithelio d cell granulomas in rabbits and guinea pigs after the intracutaneous injection of Lipovittelin, or ts protein or lipoid fraction, after the animals had been sensitized to these materials by repeated intraperitoneal injections.

Other clinical observations also incriminate immunochemical reactions in a general consideration of sarcoidosis. Recurrent colitis, an occasional pleuritis, a transitory polyarthritis, and th rarer sarcoid uveitis and thyroid tis may all be considered as accompany ng lesions, and in this respect equ valent to erythema nodosum

Complementing these clinical observations are a series of *pathological-anatomical features* prevalent in sarcoidosis which similarly n dicate that the total disease tat is elicited not by one causative agent but rather by al-





Fig. 3. Typical glomerulonephritis and interstitial nephritis in sarcoidosis. Progressive hyalinization of the Bowman capsule and obliteration of the glomerulum. R. Hans, 22 years old. MB 2811/50 St. G.

lergic phenomena. Whereas bacterial infections always cause reactiv swelling in the draining ipsilateral lymph nodes, an antigen-antibody response is always seen as bilateral symmetric lymph nodes swellings, as in the case in sarcoidosis. The second example of the presence of allergic phenomena was noted by Teilum in 1948 (19) when he observed that hyalinosis (paramyloidosis) of the reticulo-endothelial system is consistent characteristic of sarcoidosis. Upon histological examination hyaline lumps or membranes are seen between, and appear to encapsulate the sarcoid granulomas which also undergo progressive and total hyalinization (Fig. 1 and 2). The validity of attributing hyalinosis to an immunological reaction is based, according to Teilum, on

1. The development of hyalinosis following small-pox inoculation.
2. The development of sarcoidosis following BCG inoculation.

The hyalinosis of the granulomas in the reticulo-endothelial system is similar to, and may be equated with, those changes seen in the sarcoid kidney. The latter as described by Teilum in 1951 (20) entail the appearance of wire loops glomeruli and subcapsular hyaline secretions. Löfgren observed these typical changes in hypercalcemic cases, in which the intensity of the hyalinosis paralleled the degree of hypercalcemia (Fig. 3). However the glomerular hyalinosis should not be regarded merely as a direct reaction to the hypercalcemia. At the Berlin meeting in 1955, I presented case of sarcoidosis with characteristic kidney involvement. The patient finally expired due to kidney insufficiency resulting from progressive hyaline destruction of the glomeruli. The wire loop glomeruli in this case were very similar to those seen in cases of lupus erythematodes, thus giving further evidence to support the theory that the glomerular transformations are caused by antigen-antibody reactions (Fig. 4).

One of the most important pieces of evidence substantiating the presence of actively participating immunological processes during the course of sarcoidosis is its simultaneous appearance in cases with periarteritis nodosa. The latter may present the histological picture of an unspecific fibrinoid necrosis of the vascular walls with histocytic encapsulation of the necrotic area, or it may be characterised by the preferential destruction of the elastic membrane and tunica media with their replacement by epithelioid cells and granulation tissue (Fig. 5). The first observation relating to the above theory was made by H. R. Staehelin in Basle in 1942. A 20-year old woman, after recovering from mild case of polyarteritis, suffered fatal septic fever which was clinically suspect as miliary tuberculosis. Autopsy findings revealed disseminated epithelioid granulomas in the lungs, kidneys, spleen, and central nervous system with an accompanying classical periarteritis

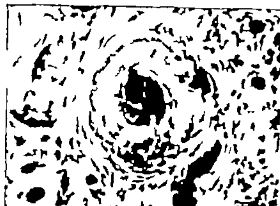


Fig 4 Typical glomerulonephritis and interstitial nephritis in sarcoidosis. Progressive hyalinization of the Bowman capsule and obliteration of the glomerulum. Final stage. B. Otto, 59 years old. SN 436/52 St G



Fig 5. Sarcoid polyarteritis. Non caseating tubercle in the vessel wall with destruction of the elastic membrane K. Jakob, 36 years. MB 9390/63.

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The occasional occurrence of sarcoidosis with lupus erythematodes and with Addison's disease resulting from adrenocortical atrophy due to an autoimmune reaction, lends further support for the theory basing the manifestations of sarcoidosis on immunological precepts. And, the disease arresting response to cortisone therapy also strongly suggests the prevalence of allergy in sarcoidosis as cortisone obstructs antigen-antibody reactions.

Yet, the theory of the immunological nature of sarcoidosis is not completely free from difficulties as may be seen in the local spreading of sarcoidosis in the form of ependymitis which displays a bacterial type of spacial expansion. Figure 6 shows two photos from the lateral ventricles of the brain of a 40-year-old woman. The patient fell ill 10 years prior to death with a bilateral hilus syndrome. After remaining asymptomatic for some time she suddenly expired with clinically evident signs of acute hydrocephalus. The autopsy revealed large tracheobronchial lymph nodes and isolated hyalinized epithelioid cell granulomas in the lungs, meninges, and choroid plexus. The main feature of the findings in the brain ventricle consisted of the fact that opposite the mobile part of the choroid plexus, beneath the ependyma, granulomas developed and grew towards the lumen, finally rupturing the ependymal covering They evoke the image of a volcanic erruption (Fig 6)

The careful observation of the figures leads to the seemingly unavoidable conclusion that the ependymal granulomas are in reality contact metastases of the primary process originating in the choroid plexus, and that an unknown agent has been transferred from the granulomas located in the choroid plexus to the ependyma. Whether this be true or not will only be shown after the etiology of sarcoidosis is definitely and completely determined. However it is my firm opinion, based on clinical, pathological-anatomical, and experimental findings that antigen antibody reactions play a predominant role in the induction and production of the clinico-pathological disease entity recognized as sarcoidosis.

The histological differentiation of sarcoidosis from tuberculosis is not difficult when the pertinent characteristics are taken into consideration. Tuberculous lymph nodes contain tubercles which may vary remarkably in size and form. The tubercle proper may consist of an extensive field of caseous necrosis with a *surrounding wall of epithelioid* and giant cells, or it may be composed of just a



Fig. 6. Sarcoid granuloma located in the choroid plexus (left) with subependymal contact metastases (right). A. Helene, 40 years. SN 260/62. (HE stain, 90 : 1)

mass of epithelioid giant cells, or it may show any transition stage between these two extremes. Tuberculous tubercles also exhibit the tendency toward confluence and final cicatrization. The latter occurs through various stages. Collagen fibrilles initially form around the nodule. The epithelioid giant cells then undergo shrinkage and appear as fibroblasts which then secrete dense collagenous fibrillar mesh. This eventually merges with the nodular fibrous capsule and the entire structure then undergoes retraction. Of special interest is the secretion of fibrin in the boundary zone between the epithelioid cell wall and the central necrosis, which in the course of time becomes transformed into hyaline.

Contrasted to the histological pleomorphy of tuberculosis is the monomorphous or homomorphous character of sarcoidosis. The hyalinized sarcoid lymph node does not cicatrize, but rather appears as spent and exhausted derivative of an antigen-antibody reaction. This interpretation would thus explain the persistence of large lymph nodes with no apparent degree of post-reactive shrinkage.

Also present in the sarcoid granulomas are variety of significant findings which may be collectively termed inclusion bodies. These are subdivided into three types: the Schaumann or conchoid body, the asteroid body and the microcentrosomes. However, although these inclusion bodies appear quite frequently and prominently in sarcoid granulomas, no exceptional specificity or etiological significance should be unduly attached to them.

Schaumann, in 1916/17 after studying various cases of sarcoidosis, described relatively large (25—40 μ) rounded or occasionally branched *calcium granules* located primarily in giant cells (Fig. 7). They are very often lamellated in the form of concentric rings or parallel folds imitating the appearance of



Fig 4 Typical glomerulonephritis and interstitial nephritis in sarcoidosis. Progressive hyalinization of the Bowman capsule and obliteration of the glomerulum. Final stage. B. Otto 59 years old. SN 436/52 St. G



Fig 5 Sarcoid polyarteritis. Non caseating tubercle in the vessel wall with destruction of the elastic membrane. K. Jakob, 36 years. MB 9390/63.

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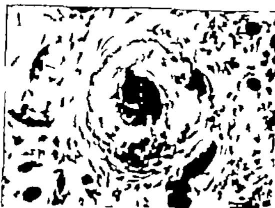


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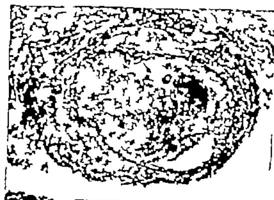


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Fig. 9. Sarcoid granuloma with centrospheres in foreign body giant cell. Lymph node. D. Hans, 44 years, MB 7884/55. 1,000 : 1

cells. Hamperl initially noted their prominent occurrence in sarcoidosis.

A study of the presented data concerning inclusion bodies leads to a seemingly sound hypothesis that each type is in reality a link in a developmental chain. This chain commences with the small centrospheres which fuse to form giant centrospheres from which the asteroid bodies evolve, which in turn metamorphose into the calcified conchoid bodies.

Conclusion

A concise yet general survey of the histological characteristics pertinent to the disease process designated as sarcoidosis yields three major points. The pathognomonic structure for the diagnosis of sarcoidosis is the sarcoid granuloma which involves all the lymph nodes, with all the granulomas showing a similar structure, size and age. The presence of inclusion bodies lends a greater degree of certainty to the diagnosis of sarcoidosis, but in no way should be considered as specific for sarcoidosis or as its causative agent. Finally the pathological anatomical picture seen in sarcoidosis gives further evidence to support the theory that the decisive causative factors for the manifestations of this disease process are the resulting effects of antigen-antibody reactions.

References

1. Bach, J., Perrin-Fayolle, M., Ledoux, A. and Biot, N.: Le Poumon et le Coeur *17*: 477, 1961.
2. Correa Pela, O.: Arch. Path. *57*: 523, 1954.
3. Fried, K. H. and Genz, H.: Tuberkulosearzt *12*: 558, 1958.
4. Hoffbrand, B. I.: Brit. med. J. *1*: 658, 1963.
5. Jackson, A. and Kass, I.: Ann. int. Med. *38*: 288, 1953.
6. Löfgren, S.: Acta tbc. scand. Suppl. *15*: 19, 1939.
7. Löfgren, S.: Beitr. Klin. Tbc. 11475, 1935.
8. Löfgren, S., Snellman, B. and Lindgren, A. G. H.: Acta med. scand. *159*: 295, 1957.
9. Löfgren, S., Ström, L. and Widström, G.: Acta paed. Suppl. *43*: 100–160, 1954.
10. Riphle, O.: Acta med. scand. Suppl. *154*: 312, 408, 1956.
11. Kalkoff, K. W. and Hotz, K. H.: Klin. Wschr. *40*: 337, 1963.
12. Klemperer, H. F. and Salley, S. N.: Bull. John Hopkins Hosp. *79*: 333, 1947.
13. Ritama, V. and Sarmal, Y.: Ann. med. int. Fenniae *38*: 188, 1949.
14. Rosenthal, S. R.: Am. Rev. Tbc. *60*: 236, 1949.
15. Seltzer, L. E. and Ehrlich, J. C.: Am. J. Med. *16*: 790, 1954.
16. Sokoloff, L. and Bunim, J. A.: New Eng. med. J. *260*: 481, 1959.
17. St. Amelin, H. R.: Virchows Arch. *309*: 235, 1942.
18. Tanoux, M.: Acta path. et microbiol. *34*: 375, 1954.
19. Teilum, G.: Amer. J. Path. *24*: 389, 1948.
20. Teilum, G.: Acta path. et microbiol. scand. *28*: 294, 1951.
21. Uehlinger, E.: Beitr. Klin. Tbk. *114*: 17, 1955.
22. Williams, M.: Ann. rheum. Dis. *20*: 138, 1961.

For further references, see Uehlinger (21)



Fig 7 Sa coidosis. Foreign body giant cell with laminated Schaumann body H Erika 29 years. MB 11270/54 420 1



Fig 8. Sarcoid granuloma in hyalinization. Asteroid body in the center of sarcoid granuloma. Lymph node. Woman, 26 years. MB. 1094/58. (Gleson stain, x 240)

sea shells (conchoid bodies) They may be seen n all organs affected by the sarcoid disease process with their numbers fluctuating greatly from case to case. They are especially numerous in cases with hypercalcemia, with the lamellated structure indicating that the initial calcium incrustation of a primary cellular inclusion served as a focus for the further precipitation of calcium phosphate.

Asteroid bodies are stellate or spicular cellular inclusions 5—20 μ in diameter and are figuratively suggestive of spiders or an open umbrella frame. They appear almost selectively in giant cells, usually of the Langhans type, and *lie within* a globular cytoplasmic vacuole (Fig 8) The center of the body occanonally contains a uniquely staining granule. The peripherally tapering rays number between 20 and 30 and appear somewhat bent. Asteroid bodies also occur in other diseases, e. g., giant cell myocarditis, thus showing their unspecific character

Centrospheres are specific central bodies or *centrioles 0.2—0.8 μ in size, characterized* by their ability to actively divide. Langhans, in 1868, observed and classically described them in connection with his giant cells as follows

Present in the giant cells, in addition to the nuclei, are many homogeneous vesicles, some sharply some poorly contoured varying in size from one-half to 3—4 times larger than a nucleus. A giant cell will contain one large or many small vesicles. I once found in a large vesicle a finely gran lar indistinctly del m ted mass. Th nuclei are displaced by the vesicle and assume a marginal position. (Fig 9) Wallgren, in 1908, also described this microcenter in epithelioid and giant

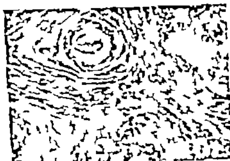


Fig. 1. Spleen in generalized sarcoidosis. Note the periarterial lamellar hyaline bands and eosinophilic, hyaline material peripherally in the individual granulomata.



Fig. 3. Periarterial fibrosis (onion-skin lesion") of the spleen in sarcoidosis. I the border zone granulomas, by fibrosis and plasmacytosis.



Fig. 2. Lamellated basophilic calcified inclusion (Schaumann body) within large multinucleate giant cell.

the marked hyalinosis in the later stages, accounting for many features in the symptomatology in cases with systemic involvement, may originate not only from proliferating cells in the granulomata, but also extragranulomatous patchy homogeneous precipitates as well as involvement of vascular walls may be seen. The prehyaline material stained with the PAS-method.

Examination of the spleen may disclose lesion, that has been presumed to be pathognomonic of disseminated lupus erythematosus, i.e., the periarterial fibrosis or so-called onion-skin lesion of the follicular and penicillary arteries of the spleen. This lesion was first observed by Sachs, and Klemperer et al. found it present in 19 of 20 cases of disseminated lupus studied by them. Kayser found it in 15 out of 18 cases and considered the lesion to be present, when the periarterial collagen was found to present at least three layers around the circumference of the vessel, producing the ppearance of concentric rings. This collagen was hyaline in most cases, and in others it consisted partially of granular eosinophilic material. Klemperer considered the lesions specific for disseminated lupus. Later this lesion was described in cases of generaliz ed sarcoidosis (Figs. 1 and 3) and certain other conditions associated with plasmacytosis and hypergammaglobulinaemia, and it was found to be related t stimulation of the immune mechanism (Teilum (13) 1948) Morphogenetically it showed similarity to deposition of amyloid or paramyloid in the same site and derived from the same periarterial parent cells. It is of interest that high incidence of periarterial fibrosis has been found also in cases of thrombotic thrombocytopenic purpura, which later has been considered representativ of diffuse vascular disease or collagenoses related to disseminated lupus erythematosus. In close relation to the hyaline or pre-hyaline lesions around the splenic essels in these conditions was often found marked accumulation of plasma cells (Fig. 3) accounting for the hyperglobulinaemia as well as "hyper globulinosis" locally in the tissue (13). This concept was supported several years later by the results of the fluorescent antibody tech-

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Morphogenesis and Development of Sarcoid Lesions Similarities to the Group of Collagenoses

Gunnar Teilum

Sarcoidosis is a chronic granulomatous disease of mesenchymal tissue of unknown etiology The granulomatous reaction may be localized, or there may be a systemic involvement of reticulo-endothelial and connective tissue. It seems that granuloma formation is preceded by a diffuse mononuclear cell infiltration and proliferation in all the sites, where granulomas are found e. g., lungs, liver skeletal muscles, myocardium, salivary glands and central nervous system. It would appear that a foreign agent may be taken up by mononuclear reticulo-endothelial cells, which react by proliferation and are changed into epitheliod cells. The lymph nodes are replaced by diffuse, evenly distributed false follicles" formed of epithelioid cells with little tendency to coalesce and never showing caseation, although it is not infrequent to find a small area of fibrinoid change in the centre. Another feature that distinguishes sarcoid from tuberculosis is the sharp demarcation from the surrounding tissue. Silver stains show a delicate reticulum, which is absent owing to destruction in tuberculosis.

Histological and cytochemical studies have revealed certain common features in distribution development and morphogenesis of th lesions in sarcoidosis and diseases associated with injury to connective tissue throughout the body i.e the so-called collagenoses. The etiology of each of the several conditions in this group is unknown, but hypersensitivity believed to be an important feature in the production of the vascular lesions of polyarteritis nodosa and thrombotic thrombocytopenic purpura, and the studies suggested, that a stimulation of immune mechanism is concerned in sarcoidosis and various types of collagenosis (Teilum, 1948)

A brief account of histological findings in sarcoidosis showing similarities to characteristic lesions in collagenoses and/or being associated with immune reactions is presented

In sarcoidosis there is characteristically a marked tendency to hyalinization to occur in the later stages of the disease, appearing as hyaline rings in th periphery of the granulomas (Fig 1) and originating from proliferating primitive reticular cells. As hyalinization progresses, the concentric hyaline rings are passing on to a diffuse hyalinosis. The tissue involved may show an extensive replacement by eosinophilic hyaline material, which may resemble amyloid, but not giving any of the characteristic staining reactions. In the lesions scattered, lamellated basophilic calcified bodies may be found, either as n clusions within large multinucleate giant cells (Fig 2) or residual granulomas or extr cellularly within completely hyalinized acellular tissue corresponding to Schaumann bodies. The histological features n cases of generalized sarcoidosis showed a marked proliferation of reticular cells ith a marked pyroninophilia as evidence of increased protein synthesis and the pre-hyaline material was produced by these cells. Interestingly



Fig. 7 Fibrinoid lesions and "hyaline thrombi" in the glomeruli in sarcoidosis.



Fig. 8. Case of generalized sarcoidosis showing wide-spread "allergic" granulomas with marked fibrinoid necrosis in the lungs.

ous should also be noted in this connection.

In another case of sarcoidosis the typical lesions in the lung were found to be associated with wide-spread peculiar granulomatous inflammation showing marked fibrinoid necrosis in the granulomas (Fig. 8) suggesting an "allergic" granulomatosis.

Recently increasing attention has been paid to involvement of the kidney by sarcoidosis. I 1935 Salvesen (11) drew attention to peculiar clinical type of renal involvement in sarcoidosis, and Löfgren et al. (1957) (7) correlating renal function and renal biopsy specimens in 16 cases of sarcoidosis found, that decidedly abnormal renal changes by studies of function and histopathology were demonstrable only in those cases, where hypercalcaemia was present.

As already mentioned also glomerular hyaline or fibrinoid lesions may be prominent feature in cases of generalized sarcoidosis. I addition to my own observation (14) (1951) with marked fibrinoid and pre hyaline changes in the glomeruli associated with necrotizing arteriolitis and normal blood pressure, Jaques (5) (1952) observed case with glomerulonephritis and arteriolitis with blood pressure 180/100 mm. Correa (2) (1954) found at autopsy the picture of subacute glomerulonephritis, Berger and Relman (1) (1955) described massive renal invasion associated with glomerular hyaline changes found at renal biopsy. There was no evidence of calcification and the serum calcium was normal.

There can therefore be little doubt that renal impairment in some cases may be due to glomerular lesions representing manifestation of the disease.

There has also been increasing awareness of rheumatic manifestations in sarcoidosis, accentuated by the report of Sokoloff and Bunim (12) (1959) of sarcoid lesions in synovial tissue obtained by needle biopsy. Manley and Shulman (8) (1960) analysed 90 cases of biopsy-confirmed sarcoidosis. The mean period of observation was 6 years. Joint involvement was significant feature in 23 % of cases. In the majority there was swelling in addition to pain. Characteristically the arthritis occurred at the onset of the disease and was often the presenting feature. It affected primarily large joints. Only one fifth had coincident erythema nodosum, and in these cases the articular disease did not differ from the remainder. A chronic type of arthritis may occur showing more protracted course with pain, swelling and deformities. The demonstration of sarcoid granulomas in the synovial tissue indicates, that the arthritis is not secondary to invasion from the bone, but represents true manifestation of sarcoidosis. Caste et al. (4) (1961) also observed such cases of sarcoidosis with diffuse symmetric



Fig 4 Vessels surrounded by sarcoid lesions.



Fig 6 Marked "hyaline" lesions in the glomerular tufts of the kidney in generalized sarcoidosis.

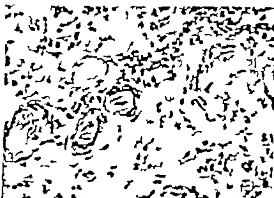


Fig 5 Arteriolar lesions associated with glomerular changes in sarcoidosis.

nique with identification of human gamma-globulin in exactly the same sites and in the same conditions, i. e. in the surroundings of granulomas in sarcoidosis, in the periarterial onion-skin lesions in amyloid and other glomerular lesions in the kidney (Mellors et al. (9) 1957 Vazquez and Dixon (16) 1957) Klein and Block (6) (1953) listed among diseases associated with bone marrow plasmacytosis rheumatoid arthritis, collagen diseases and sarcoidosis. Altogether these findings suggest morphogenetic similarities existing between mesenchymal lesions in sarcoidosis and collagen diseases associated with a stimulation of the immune mechanism with proliferation of plasma cells as well as primitive reticular cells, which may proliferate and differentiate in a fibroblastic manner resulting in a wide-spread hyalinosis.

Cytochemical studies of experimental amyloidosis and epithelioid granulomas in sarcoidosis and other conditions revealed the presence of PAS-positive material in the reticuloendothelial cells, which was considered a glycoprotein produced by the cells in response to antigenic and unspecific stimulation (Teilum (15) 1957) Obel, Lundbeck and Löfgren (10) (1962) have come to similar conclusions with the use of tissue cultures in sarcoidosis.

In contrast to the avascularity of the lesion in tuberculosis, which lessens the entry of natural antibodies to its central parts, and with larger tuberculous foci prevents it completely the granulomas in sarcoidosis are quite often supplied with vessels and may develop round vessels, which are usually not destroyed (Fig. 4)

The occurrence of arteriolar and glomerular lesions observed in isolated cases of generalized sarcoidosis, may represent another allergic manifestation of the disease.

In such a case (14) (1951) I found an extensive involvement of arterioles of kidney (Fig 5) and other organs as well as marked hyaline or fibrinoid lesions in the glomerular tufts (Fig 6) also including so-called hyaline thrombi" (Fig 7)

Arteriolar hyaline lesions of this type have been found in various allergic states, such as fulminant allergic purpura. The resemblance to the vascular changes in primary systemic amyloidosis, which is often associated with (vascular) purpura and a simple plasmacyt

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Observations on "The Sarcoid Tissue Reaction"

J. T. Kelemen

We have investigated histochemically the granulomata of sarcoidosis, of confirmed tuberculous material, and granulomata produced by Kveim antigen. The methods employed and the results obtained are shown in table I. With the methods applied no differentiation was possible among the three tissue specimen types, however the following points should be emphasized:

1. There is a strong acid phosphatase activity in all three types of granulations.
2. A protein complex containing much throzin was found in the tissues, especially in the border of the cytoplasm of giant cells.
3. A small amount of finely dispersed PAS positive material could be demonstrated in the granulomata, especially in the central areas of the giant cells. We have the impression that tuberculous giant cells contain more of this material than do sarcoidotic cells.
4. In the fresh granulomata there are some neutral and some acid lipids. In the giant cells of older granuloma only finely dispersed neutral lipids were found.
5. I embedded preparations — around the asteroid inclusion bodies — there is an optically empty area, in which phospho-

Table I. The sarcoid tissue reaction

Reactions	DDD -SH	DDD SH SS	Alk. ... SH SS	Alkaline ...	DNFB	Glenner L. ...	DMAB	Fast Blue B	Hg. Bromphenol Blue	Acid phosphat.	PAS	Baker m.	Sudan III—IV	Sudan Black B	Oil Red O
Epithelioid cells	+	+	+	+	+	+	±	+	+	++	±	—	±	±	±
Giant cells	++	++	++	+	+	++	+	++	++	++	++	+	+	+	+
Conchoid body	±	±	±	±		±		±	±						
Asteroid body	+	+	+	+	+	+++		+	+	++	+	—		—	—
Central necrosis	++	++	++	+	++	++	+	++	++		++				
Caseation/tbc	±	±	±	+	++	+++	+	+	+	—	++				

polyarthritis. Some of these were chronic as rheumatoid arthritis. The Waaler Rose reaction was always negative.

The wide-spread distribution of the lesions in generalized sarcoidosis to interstitial connective tissue and reticulo-endothelial system also including skeletal muscles, myocardium bone marrow the posterior lobe of the pituitary and hypothalamus, as well as the occurrence of arteriolar and glomerular lesions, seems strongly to suggest a generalized mesenchymal reaction, which has much in common in localization and nature of lesions with those in the collagenoses.

In the pathology of lesions in generalized sarcoidosis are included lesions such as epithelioid cell granulomas with relation to vessels and showing a typical phasic development resulting in systemic hyalinosis, periarterial hyalinosis of onion skin type, plasmacytosis and "hyperglobulinosis in the surroundings of the lesions in the active phase, extragranulomatous hyalinization, arteriolar and glomerular lesions and "allergic" granulomas with fibrinoid necrosis. In late stage of generalized sarcoidosis the wide-spread granulomas in the interstitial connective tissue of the organs involved may be replaced by an extensive hyalinosis showing marked contraction of liver and kidney

Most of these features are similar — morphologically as well as morphogenetically — to various lesions in polyarteritis nodosa, paramyloidosis, vascular purpura and disseminated lupus erythematosus. Among the clinical symptoms which may point to such a relationship should be mentioned hyperglobulinaemia and, although somewhat uncommon, purpura and hemolytic anemia. An eosinophilia from 8 to 20 % has been recorded in approximately 15 of the cases (3)

Evidently no implication can be made on a morphogenetic basis concerning specificity or common etiology

Summary

Characteristic features in the morphogenesis of sarcoid lesions are outlined, and similarities to lesions in the group of collagenoses are considered.

From a histopathologic point of view generalized sarcoidosis is a systemic granulomatous disease of mesenchymal tissue associated with a stimulation of the immune mechanism.

The typical phasic development of granulomas resulting in a systemic hyalinosis is related to an active cellular response of PAS-positive reticulum cells, while proliferating plasma cells account for the hyperglobulinaemia.

The systemic distribution of sarcoid lesions in mesenchymal tissue, the phasic development, as well as the nature of various lesions observed in such cases (e. g "hyperglobulinosis" plasmacytosis, onion-skin lesions" allergic" granulomas and arteriolar and glomerular lesions) suggest a generalized mesenchymal reaction which seems to have much in common with various lesions in the group of so-called collagen diseases

References

1 BERGER, K. W and RELMAN A. S New England J Med. 252 44 1955.

2. CORREA, P Arch. Path. 57 525 1954

3 CUMMINGS, M. M and MICHAEL, M., jr Disease-a Month, May 1960

4 COSTE, F et al. X Congres internat. Reum. Roma 1961 Comunic. Vol. II pe. 1098.

5. JAQUES, W E. Arch. Path. 53 558, 1952.

6. KLEIN H and BLOCK, M. J Hematol. 8. 1034 1953

7 LÖFGREN S., SNELLMAN B. and LINDGREN A. G H. Acta med. scand. 159 295. 1957

8. MANLEY K. A and SHULMAN L. E. Arthritis and Rheum. 3 455 1960

9 MELLORS, R. C. et al. Am. J P th. 33 613 1957

10 ODEL, A. L., LUNDBECK, H. and LÖFGREN S. Acta path. et microbiol. scand. 55. 407 1962

11 SALVESEN, H. Acta med. scand. 86 127 1935

12. SOKOLOFF L. and BUNIM J J New England J Med. 260 841 1959.

13 TEILUM, G Am J Path. 24 389, 409 1948.

14 TEILUM G Acta path. et microbiol scand. 28. 294 1951

15. TEILUM, G Am. J Path. 32 945, 1956.

16. VAZQUEZ, J J and DIXON, F J Lab. Invest. 6. 205, 1957

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Contribution to the Origin, Development and Experimental Production of Laminated Calcinosiderotic Schaumann Bodies

F. ŽÁK

In our work, we aimed at making contribution towards the elucidation of the origin of the characteristic calcinosiderotic Schaumann bodies which are sometimes considered to be specific for sarcoidosis. By analyzing bioptic and necroptic material from the 2nd Anatomicopathological Institute as well as experimental material, we were able to demonstrate almost with certainty that laminated Schaumann bodies occur also in tuberculous granulomas.

In specific granulomas, however, we mostly find fully developed Schaumann bodies. From these observations it is generally not possible to draw any conclusion on the mode of their development. It is only in rare single cases that close relationship is observed with calcified elastic fibres or calcified capillaries. We have had the opportunity however of observing other developmental mechanisms of Schaumann bodies in tuberculosis, and we have succeeded also in producing them experimentally. In human tuberculous granulomas, in addition to typical giant Langhans cells, we have found, for instance, some giant cells with phagocytized, needle-shaped, elongated crystals (60—120 μ in length), which were readily soluble in lipoid solvents. The crystals gave positive Feigen reaction with cholesterol esters. With polarized light the crystals were birefringent and their index of refraction and of double refraction was about 1.5 and about 0.02 respectively which, according to Winchel tables, would be characteristic of cholesterol derivatives. These crystals induced tissue reaction which eventually resulted in the development of laminated Schaumann inclusion bodies. At first, the crystals are bordered by narrow hem of material which gives a positive reaction for acid mucopolysaccharides, as can be seen on sections stained according to Hale-Müller (Fig. 1). In some places the border around the crystalline structures was larger and gave also a positive reaction for iron. At the outset birefringent crystals persisted in the centre of the developing structures. It was only during the later developmental phases that double refraction disappeared and then larger inclusion bodies with laminated structures were seen (Fig. 2). In the final phase dense laminated bodies were found corresponding to the well-known Schaumann inclusion bodies.

We succeeded in observing experimentally another mode of development of Schaumann bodies. We have followed the sequence of events, in 61 head of cattle, from 7 days up to 3 years after subcutaneous inoculation of Czechoslovak M-vaccine against tuberculosis.

One week after the vaccination large clusters of Mycobacteria, surrounded by leucocytes, were seen. After four weeks, clumps of

lipids could be demonstrated when using Baker's method

6 The asteroid bodies consist of complex protein, rich in tirozin, with strong acid phosphatase activity There is more tirozin in the asteroid inclusion bodies than in the cytoplasm of the giant cells.

7 It may be of interest to note, that the protein-lipid and enzyme content of the sarcoidosis granulation and that of the tissue reaction caused by Kveim antigen, appear to be almost identical histochemically

Our data concerning the inclusion bodies are based on only a few cases, since the occurrence of inclusion-bodies has been comparatively rare in our material. This is probably due to the fact that we dealt with early cases, as the disseminated form of sarcoidosis is relatively infrequent in Hungary

Lipids were investigated histochemically only in sarcoidosis and Kveim specimens, but not in tuberculosis.



Fig. 5. Compact calcifications of tuberculous granulomas, eight months after vaccination. Stained with Kossa method for an indirect determination of calcium (180)



Fig. 6. Laminated calcinosiderotic Schaumann bodies in subcutaneous granulomas of cattle, three years after vaccination. Stained with hematoxylin-eosin (210).

acid-fast rods were present in giant Langhans cells within the epithelioid granulomas which were formed. I areas with clumps of bacteria and phagocytized Mycobacteria it was possible to simultaneously observe an accumulation of PAS-positive material (Fig. 3) and, in sections impregnated according to Kossa, discrete calcification, both extracellular and within the giant cells, was noted (Fig. 4). During the subsequent months the calcifications became increasingly marked and more compact, as is evidenced by section obtained from the vaccination site after eight months and stained according to Kossa (Fig. 5). After tw or three years the calcifications were transformed into laminated Schaumann bodies which were found in the epithelioid granulomas, either extracellularly or in some places, in giant multinucleated cells (Fig. 6). I some calcinosiderotic structures or in their vicinity aggregates of small, markedly birefringent crystals were seen with polarized light. These crystals were insoluble in lipoid solvents, but soluble in diluted mineral acids.

Measurements of the index of refraction, obtained by the immersion method, varied between alpha 1.4868 and gamma 1.6496. This, and the high index of double refraction 0.1628, were characteristic of hexagonal modification of calcium carbonate (calcite) (Fig. 7).

I scars after vaccination against tuberculosis in children, w found also epithelioid granulomas which sometimes contained small Schaumann bodies (Fig. 8). The bodies showed laminated structure and, in addition to positive reaction to calcium, gave also positive reaction to iron. W conclude that these structures develop in similar way to those observed in cattle, and that even epithelioid granulomas should not be considered as representing local manifestation of sarcoidosis developing after vaccination against tuberculosis.

I summing up the results of our observa



Fig 1 Deposits of acid mucopolysaccharides around needle-shaped cholesterol crystals, stained ccording to *Hale-Muller for* cid poly saccharides (× 375)



Fig 3 PAS-positive material at the site of phagocytized bacteria, four weeks after vaccination. Stained according to MacManus (× *180*)



Fig 2 Laminated arrangement of structures around crystals *in* specific granulation tissue. Stained ccording t Hale-Muller for acid mucopolysaccharides (× 200)



Fig 4 I itial calcifications in specific granulomas, four weeks fter vaccination. An indirect determination of calcium by Kossa's method (× *180*)



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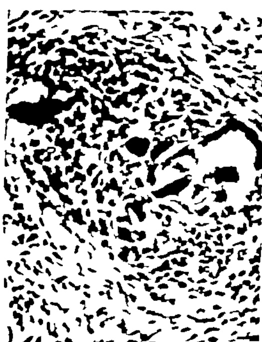


Fig. 3. PAS-positive material [illegible] of phagocytized bacteria, four weeks after vaccination. Stained according to McManus ([illegible] 180



Fig. 2. Laminated arrangement of structures around crystals in specific granulation tissue. Stained according to Hale-Müller for acid mucopolysaccharides (× 200)



Fig. 4. Initial calcifications in specific granulomas, four weeks after vaccination. An indirect determination of calcium by Kossa method (× 180)



Fig. 5. Compact calcifications of tuberculous granulomas, eight months after vaccination. Stained with Kossa method for an indirect determination of calcium (180)

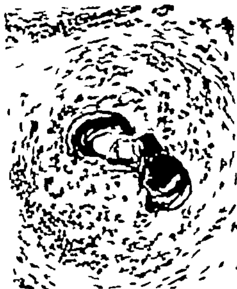


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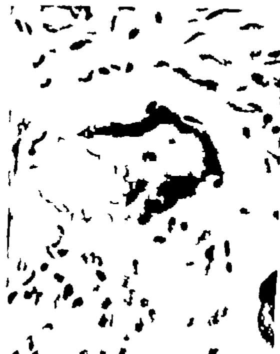


Fig 7 M kell l ef gent k t rystal gia t lt ud ted ll 3 y rs fter t on Inspected by p larized l ght 400



Fig 8 A small calcinosiderotic inclus on bod w th n gia t cell from ubcutaneous granuloma, found in hild fter BCG accination. St ined w th hemat xylin eosin (450

tion bel es that characteristicall l m inated k inos lerot bodies which morpholog cally sim la may develop for arious r son In the in t l phases a com mon f ature i the accu m l tion of so ub stan g g a posit reaction to muco polysa charides These substances tak up ak m nd iron salt and graduall dense cal inosiderot tructures develop In older compact lcific tions th react on to muco polysa harides is no longer posit After a further lapse of tim lam nated Scha m nn bodies develop by means of physicochemi cal transformation a observed n Liesegang circles. Schaumann bodies are usuall not birefringent Only the small calcit crystals are markedly birefri gent which develop in some places by a secondary crystallization within the structure of the Schaumann bodies and in amorphous clods of calcium, or n thei icinity

Calcinosiderotic Schaumann bodies represent the ult mate phase of tissue reaction gr nulom s, which involves the formation of small, not easily degraded, calcified struc tures These bodies are n t pathognomonic for sarcoidosis and re also found in tuber ulosis, n ariably associated, of course w th tissu hanges of a markedly proliferati e cha acter Furthermore they are sometimes found ii mall scars after th accination of human subjects gainst tuberculosis, and can be experimentally nduced in cattle by a subcuta eous pplic tion of the M-vaccine pre pared from murine Mycobacteria, hich are t pathogeni for human beings and cattle

References

1 Winch L, A W The Optical Properties of Organic Compounds. Acad Press, New York 1954

2 Zák, F Acta Universitatis Carolinae Medica 7 573—558, 1959

3 Zák, F and b ..a, L. Pathologia et Microbiologia 23 456—471 1960.

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Tissue Reaction in Sarcoidosis

A Report on Examinations Using the Coverslip Method

F Mlczoch and J Kohout

In 1961 w reported for the first time on our experience in the examination of arious diseases with the coverslip method introduced by Rebuck 1947 (1) The following is report on our findings in 102 proven cases of sarcoidosis.

Method

The skin on the anterior surface of the thigh is leaned with alcohol and the place here the lesion to be made is sprayed with chlorethyl.

Using sterile Thiersch-knife or scalpel sion lesion of bout 15 2 mm and depth of bout 1 mm is made. The depth desired is indicated by the ppearance of fine bleeding points when the papillary layer of the corium reached.

By means of sterile forceps the skin lesion is covered by rather thick sterile coverslip of bou 24 22 mm. The coverslip is fixed to the skin with adhesive tape and is covered with piece of sterile gauze. The coverslip is removed 2, 4 5, 7 10 12, 14 and 24 hours after the lesion was made and each time is replaced by new coverslip. The coverslips, with the secretions turned upward, are fastened with cedar-oil on slides, dried in the open air stained like bloodsmears, and then examined

This series of coverslips provides specimens of different stages in the tissue reaction to the wound. The course of this reaction varies in different diseases

Normally from 2 to 4 hours after the incision was made the specimens show almost exclusively granulocytes sporadically monocytes, rarely eosinophils. However 5, 7 and 10 hours after the lesion was made the picture has changed. More and more mononuclear cells ppear at first with rather round nucleus and dense chromatin structure. After while the nuclei of these cells become larger and their chromatin pattern is finer As the same time the cytoplasm increases Nucleus and cytoplasm become indented and, thus, polymorphous cells are observed. Between 12 and 14 hours after the incision was made cells of this type amount to bout 50 of all the cells in the specimen. Their number increases in specimens obtained later but the nuclei again assume their round shape. I some cells, as result of mitotic division, there re 2 3 and more nuclei. Details re given in our former reports (2)

The curv of lymphohistiocytes t corresponding intervals of time shows different courses, which w term "tissue-reaction (Fig 1)

1 The normal course.

2. A hyperactive course.

Here the number of lympho-histiocytes which ppeared 5 and 7 hours after the incision was made often amounted to more than 20 and 40 respectively of all cells in the specimen. The eosinophils were also frequently increased in all of the specimens. The more rapid and vigorous the rat of trans-

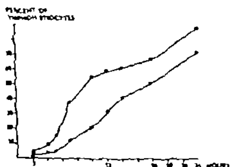


Fig 1 o——o——o Hyperactive lymphohistiocytic reaction in case of sarcoidosis. I the beginning steep rise of the curve.
——x—— Normal lymphohistiocytic reaction slow steady rise of the curve.

formation the larger was the number of multinuclear giant cells (up to 10) This course is most striking in cases of sarcoidosis.

3 A hypoergic course, often observed in malignant tumours and characterized by the appearance of only a moderate number of lympho-histiocytes

We examined 102 cases of sarcoidosis with the coverslip method and a full report will appear elsewhere

1 Tissue-reaction and stage of disease

The more acute and recent the cases of sarcoidosis the more indications of hyperactive tissue-reaction were observed. All the ten cases with erythema nodosum (Löfgren's syndrome) were among the 27 patients with hyperactive tissue-reaction of stage I. Most cases with normal or attenuated tissue reaction have reached stage III The tissue reaction is seen also in the majority of cases with only extrapulmonary lesions.

2 Tissue-reaction and patients age

The highest percentage of cases with hyperactive tissue-reaction was observed in patients up to 40 years of age The hyperactive tissue-reaction then slowly decreases and is lowest at the age of 55—65 It is between these ages that the highest percentage of cases with normal or attenuated (hypoergic) reactions is found

3 Tissue-reaction and serum protein changes

As a rule cases with attenuated tissue-reaction have a negative Latex test and their total serum protein is mostly low

The majority of cases with positive Latex test have also a hyperactive tissue-reaction most often the gamma-globulins are increased

We have little doubt that in these relations between tissue-reaction, serum protein gamma-globulins and positive Latex-test represent the common principle of sarcoidosis. As yet it cannot be determined whether the obvious discrepancies, the lack of correspondence between hyperactive tissue-reaction and increased serum protein fractions, are of pathogenic importance. These observations may be due to inadequate methods of examination

4 Other indications of changed reactions in sarcoidosis

We observed that lesions, where the coverslip method was used, healed more rapidly than similar lesions in other patients. Our sarcoidosis patients told us that they had noticed that their other wounds healed rapidly for instance after operations.

Löfgren has already mentioned positive qualities of character found in sarcoidosis patients. We have often observed this attitude in our patients. By and large patients with sarcoidosis have an understanding of their illness. They are open-minded regarding necessary diagnostic procedures and treatment.

Another indication in this direction is seen in an occupational survey of sarcoidosis patients. More than 25 of them belonged to intellectual professions.

5. Influence of therapy on tissue-reaction

During treatment with corticosteroids the hyperactive curve of the lymphohistiocytes in sarcoidosis approaches normal. As a rule this course is a sign of improvement from the clinical point of view

Summary

The report deals with examinations where the coverslip method was applied in 102 cases of sarcoidosis. A high percentage of these cases showed a characteristic hyperactive tissue reaction. A sign of the hyperactive reaction is the early appearance, and increased number of cells termed "lympho-histiocytes" on the coverslips. There is a certain relation between tissue reaction and other findings, this applies especially to changes in serum-protein and to the Latex-test. During treatment with corticosteroids the tissue-reaction was normalized.

References

1 Rebuck, J W Amer J clin. Path 17 614 1947
2. Miczoch F and Kohout J Klin. Wochenschr 40 99 1962

From the State Veterinary Medical Institute and St. Göran Hospital, Stockholm Sweden

Pathogenesis of Hyaline Formation in Sarcoidotic Lymph Nodes[1]

ANNA LISA OBEL and SVEN LÖFGREN

Most of those who have studied the histopathology of sarcoidosis consider that the granulomas heal fairly rapidly by fibrous induration with strong tendency towards hyalinization (Ricker and Clark 1949 Longcope and Freiman 1952, Zettergren 1954 Sorger and Taylor 1961 and others) Quite another hypothesis to explain the pathogenesis of the hyalinization has been put forward by Teilum (1948) According to him, the hyaline deposits represent primary activity of RE-cells. One feature which he found in common for such diseases as sarcoidosis and lupus erythematosus disseminatus as well as others was an association between hyperglobulinemia and paramyloidosis or hyalinosis in the RE-system. These diseases were also accompanied by periarterial hyaline rings about arterioles in the spleen and other organs ("onion skin lesion") All these phenomena were interpreted as representing an immunity reaction, allergic hyperglobulinosis in the RE-system, and as sign that sarcoidosis is related to the collagenoses (Teilum 1956)

Material and Methods

Lymph nodes from 17 patients with pulmonary sarcoidosis were examined. In 12 of the patients the sarcoidosis was in an early stage with bilateral hilar adenopathy and in five of them combined with a fresh mottling of the parenchyma. Four of the patients with early sarcoidosis had erythema nodosum initially I five patients the sarcoidosis was chronic with more or less pronounced fibrosis.

The lymph nodes were removed either from the scalenus region or from the mediastinum according to the method of Carlens. They were fixed in 10 per cent neutral formol and in Carnoy solution and stained with hematoxylin and eosin, van Gieson stain, PAS, toluidine blue, methyl-green-pyronin according to Unna and Pappenheim, sulphated by Kramer and Windrum method, Sudan black B, Hale colloidal iron, alcian blue, phosphotungstic acid and hematoxylin, and by Gomori silver stain.

Microscopical examination

All the lymph nodes had the characteristic changes of sarcoidosis with multiple, uniform, epithelioid-cell granulomas without necrosis. The ppearance was the same in the acute and chronic cases old and fresh granulomas were seen together I all but two of the lymph nodes there was hyaline material around the granulomas and in six of them there were also small hyaline islands not associated with the granulomas.

The formation of hyaline could most easily be followed in the isolated deposits. Focal

[1] This study was supported by grant from the Syskonen Wesséns Stiftelse"

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Microscopical examination

All the lymph nodes had the characteristic changes of sarcoidosis with multiple, uniform, epithelioid-cell granulomas without necrosis. The appearance was the same in the acute and chronic cases old and fresh granulomas were seen together. In all but two of the lymph nodes there was hyaline material around the granulomas and in six of them there were also small hyaline islands not associated with the granulomas.

The formation of hyaline could most easily be followed in the isolated deposits. Focal

[1] This study was supported by grant from the Svenska Wenström Stiftelse

formation the larger was the number of multinuclear giant cells (up to 10 %). This course is most striking in cases of sarcoidosis.

3. A "hypoergic" course, often observed in malignant tumours and characterized by the appearance of only a moderate number of "lympho-histiocytes".

We examined 102 cases of sarcoidosis with the coverslip method and a full report will appear elsewhere.

1. Tissue-reaction and stage of disease

The more acute and recent the cases of sarcoidosis the more indications of hyperactive tissue-reaction were observed. All the ten cases with erythema nodosum (Löfgren's syndrome) were among the 27 patients with hyperactive tissue-reaction of stage I. Most cases with normal or attenuated tissue reaction have reached stage III. The tissue-reaction is seen also in the majority of cases with only extrapulmonary lesions.

2. Tissue-reaction and patients' age

The highest percentage of cases with hyperactive tissue-reaction was observed in patients up to 40 years of age. The hyperactive tissue reaction then slowly decreases and is lowest at the age of 55—65. It is between these ages that the highest percentage of cases with normal or attenuated (hypoergic) reactions is found.

3. Tissue-reaction and serum protein changes

As a rule, cases with attenuated tissue-reaction have a negative Latex test and their total serum protein is mostly low.

The majority of cases with positive Latex test have also a hyperactive tissue-reaction; most often the gamma-globulins are increased.

We have little doubt that in these relations between tissue reaction, serum protein, gamma-globulins and positive Latex-test represent the common principle of sarcoidosis. As yet it cannot be determined whether the obvious discrepancies, the lack of correspondence between hyperactive tissue-reaction and increased serum protein fractions, are of pathogenic importance. These observations may be due to inadequate methods of examination.

4. Other indications of changed reactions in sarcoidosis

We observed that lesions, where the coverslip method was used, healed more rapidly than similar lesions in other patients. Our sarcoidosis patients told us that they had noticed that their other wounds healed rapidly, for instance after operations.

Löfgren has already mentioned positive qualities of character found in sarcoidosis patients. We have often observed this attitude in our patients. By and large, patients with sarcoidosis have an understanding of their illness. They are open-minded regarding necessary diagnostic procedures and treatment.

Another indication in this direction is seen in an occupational survey of sarcoidosis patients. More than 25 % of them belonged to intellectual professions.

5. Influence of therapy on tissue-reaction

During treatment with corticosteroids the hyperactive curve of the lymphohistiocytes in sarcoidosis approaches normal. As a rule this course is a sign of improvement from the clinical point of view.

Summary

The report deals with examinations where the coverslip method was applied in 102 cases of sarcoidosis. A high percentage of these cases showed a characteristic hyperactive tissue reaction. A sign of the hyperactive reaction is the early appearance, and increased number of cells termed "lympho-histiocytes" on the coverslips. There is a certain relation between tissue reaction and other findings; this applies especially to changes in serum-protein and to the Latex-test. During treatment with corticosteroids the tissue-reaction was normalized.

References

1. Rebuck, J. W.: Amer. J. clin. Path. *17*, 614, 1947.
2. Mlczoch, F. and Kokout, J.: Klin. Wochenschr. *40*, 99, 1962.

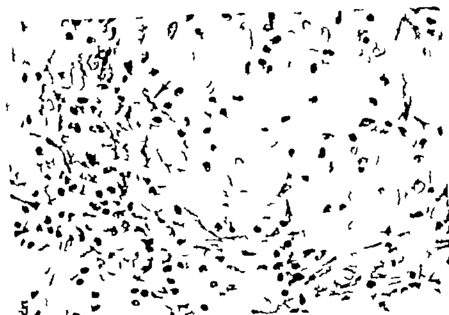


Fig. 5. P 1601/59. Sarcoidosis, lymph node. Hy aline material in RE-cells the periphery of granuloma. Hyaline-producing cells lso lie be tween the epithelioid cells within he granuloma (arrow) PAS, 500

proliferation of RE-cells gav islands of cells with large, light nuclei. The cytoplasm of these cells contained numerous trongly PAS-positive granules which ppeared t coalesce to form larger hyaline-like, less strongly PAS-positive masses in the cells (Fig 1) Some of the RE-cells could be entirely filled with hyaline ma erial. The material also began to accumulate along reticulin fibres. When hy alinisation had progressed sufficiently the hyaline appeared as extracellular masses with somewhat fibrillar tructure Cell nuclei enclosed in the hyaline appeared to undergo gradual lysis and ultimately disappeared (Fig 2) At the edges of the larger hyaline deposits there were few surviving RE-cells with the strongly PAS-positive granules which seem to be the initial phase in the process. Immedi- ely surrounding the hyaline deposits and particularly during the ini ial phase there was often an accumulation of plasma cells. The proliferating RE-cells, however did not have detectable pyroninophilic phase.

Swollen RE-cells with PAS-positiv cyto-plasmic granules appeared apparently quite early at the periphery of the fresh granulomas (Fig. 3) Just as was the case for the isolated hyaline deposits, the granules coalesced to result in deposition of hyaline masses along reticulin fibres and the formation of coarse strands (Fig 4) These strands lay concentri-cally around the granulomas and often were confluent with those surrounding adjacent granulomas (Fig. 5) Hyaline-producing cells also ppeared within the granulomas and the epithelial cells gradually became enmeshed in network of hyaline strands As the granu-lomas aged, the network of argyrophilic fibres in them became denser I the later stages, collagen fibres ppeared to develop from the hyaline ma erial withou apparen fibroblast activity



Fig. 1. P 1601/59. Sarcoidosis, lymph node. Focal proliferation of RE-cells unrelated to granuloma. The cytoplasm of the proliferating RE-cells contain numerous PAS-positive granules which in places (arrow) have coalesced to form larger masses. PAS 500



Fig. 2. P 6375/58. Sarcoidosis, lymph node. Hyaline deposits unrelated to granulomas. Some of the deposits enclose nuclear remnants (arrow). PAS 500



Fig. 3. P 1601/59. Sarcoidosis, lymph node. Periphery of fresh granuloma with swollen RE-cells containing PAS-positive material in the cytoplasm (arrows). PAS 500 ×

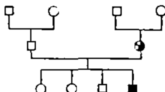


Fig. 4. P 1601/59. Sarcoidosis, lymph node. At the edge of granuloma RE-cells are arranged concentrically and contain homogeneous, PAS-positive material in their cytoplasm. PAS 500

Dr Siltzbach As clinicians we are more and more conscious of our great need for purposes of prognosis and treatment to estimate the duration of sarcoidosis; but, aside from exceptional instances, the pathologist has not found it possible to help us much in this regard.

I enjoyed these valuable discussions but I did note one omission, a subject which was mentioned only in passing by Prof. Teilum. I refer to "fibrinoid necrosis" which is so often seen within the epitheloid cell tubercle in sarcoidosis in contrast to the initially external location of the paramyloid hyaline deposits to which fibrinoid necrosis appears to be unrelated. Only later in the course of healing of sarcoidosis do the fibro-hyaline masses invade and eventually replace the epithelioid cell masses. I would suggest that the presence of fibrinoid necrosis within the sarcoid tubercle is a relatively fresh phenomenon although I grant that once fibrinoid necrosis is established, it may persist for years within the tubercle and especially within coalescent tubercles. I have always supposed, too, that the presence in any tissue of substantial paramyloid or fibro-hyaline deposits around or encroaching upon the sarcoid tubercle indicates a process of at least one to two years duration provided that the patient has not received corticosteroid therapy. I think it might now be helpful for us to re-examine the lymph nodes and other tissues removed from patients with early sarcoidosis shortly after the onset with erythema nodosum or recent hilar adenopathy. If the tissues from these early cases do not, in fact, exhibit substantial paramyloid deposits and my experience thus far indicates they do not, and if, in addition, fibrinoid necrosis within the tubercles is relatively frequent, I think we could then say with greater assurance that the presence of easily recognizable amounts of paramyloid indicates sarcoidosis of some standing. Whenever I am presented with a chest film of a patient with sarcoidosis showing disseminated pulmonary lesions and there is no recent film available to help me date the lesions, I examine the biopsies of the involved tissues and if they show peripheral fibrosis and hyalinization around some or all of the tubercles, I consider that the case is no longer a fresh case. Dr Obel has just shown us paramyloid masses in small amounts even in relatively early cases but I am referring to more pronounced hyalinization and fibrosis.

Dr Jönssen Es bestehen eine Reihe von klinischen und histologischen Ähnlichkeiten zwischen der Sarkoidose und der Gruppe der sogenannten Kollagenosen. Ich glaube jedoch nicht, daß auch ätiologische Zusammenhänge bestehen. Ich verfüge zwar über eine Beobachtung, bei der in derselben Familie der Sohn an einer Hilussarkoidose und die Mutter an einem typischem Lupus erythematodes disc. faciei leiden (Abb. 1). Vermutlich handelt es sich jedoch um ein zufälliges Zusammentreffen beider Erkrankungen in einer und derselben Familie. Das gilt auch für eine weitere Familie, in der Mutter und Sohn an einer Sarkoidose, eine Tochter (bzw. Halbschwester) jedoch an einer Spondylitis ankylopoetica leiden (Abb. 2).



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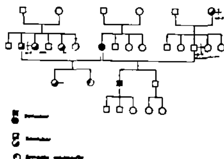


Abb. 2

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Discussion

Amyloid and similar hyaline substances are most probably related in their chemical composition and origin. Whether amyloid is formed intra- or extracellularly has long been a point of debate. The older and generally accepted hypothesis is that it represents extracellular precipitation of blood protein, possibly the result of an antigen-antibody reaction. The studies of Teilum (1956) and Hjort and Christensen (1961), however, have demonstrated that amyloid is actually formed intracellularly in RE-cells. As the first phase in the process, the proliferating RE-cells become pyroninophilic before the PAS-positive material appears. Although a pyroninophilic phase was not observed in the sarcoidotic lymph nodes, the formation of the hyaline followed the pattern described by Teilum and by Hjort and Christensen for experimental amyloidosis. The formation of amyloid is considered to be an immunopathological reaction which develops when the defences of the body are exhausted. Some infections are known for their propensity to induce the prerequisites for the formation of hyaline. Plasmacytosis in mink, for example, is a virus disease and has much in common with multiple myeloma in human beings. Hyaline deposits were observed in 42 per cent of a series of 36 autopsied mink (Obel 1959). The high incidence of hyalinosis in sarcoidosis may be a sign that the disease affects only human beings with a tendency towards abnormal immunological responses or that the disease is caused by an agent which readily induces abnormal responses.

Summary

The pathogenesis of hyaline formation in sarcoidosis was studied in lymph nodes excised from 17 patients. The microscopical appearance of all the lymph nodes was typical of sarcoidosis. Even in acute cases there were signs of healing with fibrosis and hyalinization; the duration of the disease could not be determined from the microscopical appearance.

Hyaline is formed intracellularly in proliferating RE-cells around the granulomas and in isolated islands in the lymphatic tissue. The first change is the appearance of PAS-positive granules in the cytoplasm. These granules then coalesce to form large homogeneous masses which often contain cell nuclei. The hyaline is not metachromatic but resembles amyloid structurally. Histochemical reactions for muco- or glycoprotein were obtained with the hyaline.

Literature

1. Carlens, E. Dis. Chest 36: 313. 1959.
2. Hjort, G. H. and Christensen, H. E. Acta rheum. scand. 7: 62—64. 1961.
3. Longcope, W. T. and Freiman, D. G. A study of sarcoidosis. Medicine 31: 1—132, 1952.
4. Obel, A. L. Amer. J. vet. Res. 20: 384—393. 1959.
5. Ricker, W. and Clark, M. Amer. J. clin. Path. 19: 725—749. 1949.
6. Sorger, K. and Taylor, W. A.: Arch. Path. 71: 35—43. 1961.
7. Teilum, G. Amer. J. Path. 24: 389—407. 1948.
8. Teilum, G. Amer. J. Path. 32: 945—960. 1956.
9. Teilum, G. Nord. Med. 7: 1137—1141. 1958.
10. Zetterqren, L. Lymphogranulomatosis benigna. Almqvist & Wiksell, Uppsala 1954.

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8. Teilum, G Amer J P th. 32 945—960 1956.

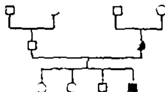
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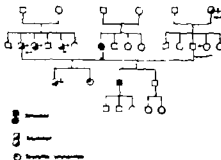


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Literature

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6. Sokoloff, K. and Taylor, W. A.: Arch. Path. 71: 35—43, 1961.
7. Teilum, G.: Amer. J. Path. 24: 389—407, 1948.
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From the National Bacteriological Laboratory and St. Goran Hospital, Stockholm, Sweden.

The Occurrence of Natural Antibodies in Sarcoidosis

Lars Olof Kallings and Sven Löfgren

Introduction

It is well known since the early days of bacteriology that fresh serum from normal healthy individuals possesses bactericidal activity against a variety of bacterial species. Later it was found that fresh normal serum also has the ability to neutralize a series of different viruses. The bactericidal and virus neutralizing activity is destroyed by heating serum to 56° C. This activity is therefore referred to as the thermo-labile in contrast to the heat-stable activity caused by ordinary antibodies and by a variety of non-specific serum compounds. Whereas ordinary antibodies are formed as a direct consequence of stimulation with a known antigen, the thermo-labile activity cannot be directly related to an immunization process. The thermo-labile activity has therefore often been classified as non-specific and has been related to the so-called natural resistance to infection (19).

In 1954, the interest in these phenomena was renewed after the isolation by Pillemer of a new serum factor which was given the name properdin (15). The discovery of properdin caused a considerable amount of excitement and much work was done on this substance. It had long been known that complement was necessary for the thermo-labile activity of normal serum. Complement alone cannot perform the normal serum activities. It is only together with another serum factor that complement displays activity. According to Pillemer this factor was properdin. Adsorption of properdin from serum abolished the bactericidal and virus neutralizing effect, addition of properdin restored it. In addition to the factors mentioned, *magnesium ions were necessary*. Complement, properdin and magnesium were called the properdin system. This system was found to cause a large number of diverse effects. The bactericidal effect was directed particularly against gram-negative organisms (20); the virus neutralizing effect was demonstrated against for instance vaccinia-variola virus, influenza and herpes virus (21, 2, 1) and also against various bacterial viruses (22, 6). The properdin system was also found to kill certain protozoa (3) and to lyse abnormal erythrocytes (4), for example erythrocytes from patients suffering from paroxysmal nocturnal hemoglobinuria.

The various activities mentioned are well established and have not been called in

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Dr JAMES W have all enjoyed the bea utiful demonstrations of sarcoid tissue shown by world experts on the ordinary light microscope, but surely the time has now come for demonstrations of sarcoid tissue by electron microscopy I cannot find electron microscopists in Europe t study sarcoid tissue because they are too busy working on cancer and ha e not yet become interested in sarcoidosis. Indeed, the only studies I ha e seen were shown to me by Dr Louis Siltzbach. I pre sume that they were done n the Mt. Sina Hospital, New York.

Dr REID I found Dr Mlczoch paper on the cellular reactions in sarcoidosis very i teresting After all, this is a disease which is characterised by accumulations of histiocytic and epithelioid cells. As well as demonstrating the ability of such cells to be mobilised, it is necessary to know that they retain their normal functions, and to test this is rather difficult. W ha e been using as functional test the bility of the patient with sar coidosis to cause the disappearance of intradermal njections of his own whole blood by con parison w th normal subjects. To summarise, the cellular response patients w th sarcoidosis to injections of their own blood as judged by time of disappearance of the bruise, is just as effective and rapid as in normals. Admittedly the endpoint is not very satisfactory but there seems no reason to doubt that patients with sarcoidosis show th same bility to produce a macrophage reaction as ordinary patients.

Dr UEHLINGER I would like to refer to a work by Spiro "To the etiology of the Besnier Boeck Disease" Ann. paed. 154 199, 1940 which may be easily correlated with the accelerated histiocytic reaction as demonstrated in sarcoidosis by the cover slip method of Mlczoch. The paper of Spiro contains a report on case nvolving sar coidosis in a 13 yea old boy complicated by the dramatically rapid development of tuberculin reaction. A spherical area of erythema and infiltration measuring 5 cm in diameter developed, after only a few hours, around the si of the tuberculin injection. Spiro denoted this acceler ated Pirquet reaction as Strohfeuerpirquet" i.e., he likened it to the quickly ignited, very volatile, consuming blaze of haystack. This reaction, when observed in sarcoidosis, can be considered the morphological equi alent to the histiocytic activation demonstrated with the cover sl p method.

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The Occurrence of Natural Antibodies in Sarcoidosis

LARS OLOF KALLINGS and SVEN LÖFGREN

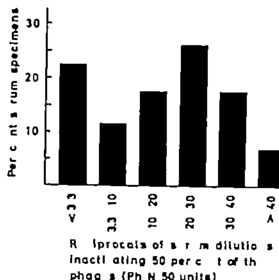
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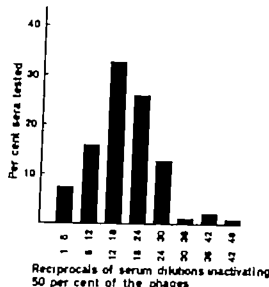


Fig 1 *Distribution of Coli T 2 neutralization titers of sera from 90 sarcoidotic patients.*

Reactions mixtures: 0.5 ml serum dilution + 0.5 ml phage suspension containing about 1×10^4 plaque forming units. Diluent 0.125 M barbiturate buffer pH 7.4 ± 0.05. Mg^{++} added to 0.0025 M. Incubated at 37° C for *two hours.*

Fig 2. *Distribution of Coli T 2 neutralization titers of normal sera from 94 healthy humans.* From KALLINGS (6)

Reaction mixtures 0.5 ml serum dilution + 0.5 ml phage suspension containing about 1×10^4 plaque-forming units. Diluent 0.125 M barbiturat buffer pH 7.4 ± 0.05 Mg^{++} added to 0.0025 M. Incubated at 37° C for *one hour*

question. There has, however been a lot of discussion concerning the nature of properdin, whether it is a single substance or is composed of a family of substances.

Many immunologists consider properdin not to be a new unique serum component but to be the same as was previously referred to as natural antibodies. This may not make people feel any happier about the situation, it is really just to call a rose by another name. However many new facts have been acquired by the studies mentioned. For instance, the natural antibodies have been found to belong to the gamma globulins showing many similarities to the specific antibodies formed early on in the course of the immunization process. These early specific antibodies, belonging to the same Cohn fraction as natural antibodies, are also dependent on complement (14) In addition, the natural antibodies have shown an unexpected specificity to various antigens. So the border-line between natural and specific antibodies no longer seems to be well defined.

The purpose of the present study was to see whether or not persons with sarcoidosis behave similarly to healthy persons as to the activity of natural antibodies. A test system of bacterial viruses was used for this investigation (5, 7)

Material and Methods

Sera. Sera were examined from 90 patients with pulmonary sarcoidosis of different stages. In all of them biopsy of scalene lymph nodes and/or skin revealed histopathologic picture compatible with sarcoidosis. In eight cases the disease was in fairly acute stage, manifested by erythema nodosum within six months prior to the serological examination. In the other cases the sarcoidotic process was subchronic or chronic, from one to about fifteen years old. In 17 cases there were besides the involvement of lungs and adjoining lymph nodes, signs of generalization. In 12 cases scar sarcoids of the skin (10) and in 5 cases history of renal involvement with hypercalcemia (11)

The sera were frozen at − 20° C two hours after bleeding and transferred to − 70 C for storage.

Neutralization test. Coli T 2 phages were used for the test system. The serum titre causing 50

TABLE I. The clinical type of sarcoidosis cases within groups without and with demonstrable natural antibodies

Level of T2 neutralizing activity (Ph N 50 units)	No. of cases	No. of acute cases (erythema nodosum)	No. of cases with signs of generalization		
			Skin	Kidneys	Total
< 3.5	20	1 (5.0 ±4.9)	6 (30.0 ±10.3)	3 (15.0 ±8.0)	9 (45.0 ±11.1)
> 3.5	70	7 (10.0 ±3.6)	6 (8.6 ±3.3)	2 (2.9* ±2.0)	8 (11.4** ±3.8)
Difference		5.0** ±6.1	21.4 ±10.8	12.1 ±8.2	33.6% ±11.7

per cent neutralization of the bacterial viruses (Ph N 50 units) was determined using a technique described in a previous paper (5). In the main, the technique involved mixing serial serum dilutions with a fixed virus dose and incubation at 37° C for two hours. The reaction was then stopped by chilling and dilution. The remaining virus activity was determined by plating the serum-virus mixture together with coli bacteria and by counting the resulting phage plaques after incubation overnight. The degree of neutralization was calculated according to the plaque numbers in two kinds of true controls where the native serum of the reaction mixture was replaced by nutrient broth and by serum, inactivated by heating to 56° C for 30 minutes.

Results

When the neutralization by normal serum of coli T 2 bacteriophages was used as a test system reflecting the activity of natural antibodies, *it was found that 70 out of 90* sarcoidotic patients possessed such activity (fig. 1). As many as 20 (22 per cent) of the sarcoidosis cases, however, lacked measurable phage neutralizing activity as compared to none among the 94 healthy *persons tested* in a previous investigation (6) illustrated in fig. 2.

On the other hand, as seen in the figures, the distribution of the titers among the 70 sarcoidosis cases showing *neutralizing* serum activity was about the same as for the healthy individuals.

In principle, the lack of *neutralizing* potency might be due to deficient complement activity as well as to disturbances in *some of the other factors participating in the* thermo-labile antimicrobial serum system. The hemolytic activity of the serum specimens without phage neutralizing effect was therefore examined as a simple test for the efficiency of the complement. It was found that all but one of the 20 sera lysed sensitized sheep erythrocytes, *i.e. exhibited at least* hemolytic complement activity.

There were no differences of the natural antibody level according to sex and age of the patients, or age of the sarcoidotic pulmonary process. Instead, a comparison was made between two groups: patients with *and patients without demonstrable natural* antibodies (Tab. I). In this respect, too, acute and chronic cases were distributed in a similar manner among the two groups: the frequency of erythema nodosum was 10.0 and 5.0 per cent, respectively. The difference is not significant.

Within the group without demonstrable natural antibodies there was, however, a higher incidence of generalized cases. Thus, *skin sarcoids were found in 30.0 per cent, and history of hypercalcemia in 15.0* per cent, compared with respectively 8.6 and 2.9 per cent in the group with a normal level of natural antibodies. These differences are not significant, however, possibly owing *to the smallness of the groups. If the total* number of generalized cases within *the two* groups is considered, the incidence figures will be 45.0 and 11.4 per cent, respectively, and the difference, 33.6 ± 11.7 per cent, is almost significant.

Discussion

The principal result of the present investigation was the finding that well over 20 per cent of the sarcoidotic cases examined lacked the phage neutralizing ability which seems to be regularly present in healthy individuals. Not only did the 94 healthy persons in the group, used as control for this investigation, possess neutralizing activity but single specimens without neutralizing activity were encountered only occasionally among an additional material of about 500 human sera tested previously and representing various physiological and pathological conditions. As a reason for the absent neutralizing effect in these occasional cases, a lacking hemolytic complement activity due to anticomplementary effect or other reasons has always been found. This seemed to offer a possible explanation also with regard to the present material, especially as anticomplementary effect has been shown to occur frequently in sarcoidosis (17). In contrast hemolytic complement activity was lacking in only one of the 20 sarcoidosis sera without demonstrable phage neutralizing potency. The inability to neutralize phages among the sarcoidotic patients might therefore be due more directly to the deficiency or inactivity of the natural antibodies.

Although titer variations of the neutralizing activity have been demonstrated, a corresponding state of non-functioning of the natural antibody system, as far as the phage neutralization is concerned, seems not to be reported in any other disease. Newborn children have, however been found also to be without the phage neutralizing activity (8). The significance of the finding in sarcoidosis is as yet obscure but may be compared with other immunological anomalies in that disease such as the "anergic" state represented by a weakening or disappearance of the tuberculin and other skin reactions of the delayed type, a phenomenon which, however, does not concern the circulating antibodies. The humoral antibody response to antigenic stimuli seems not to be affected in sarcoidosis, as surveyed by Scadding at the present conference (18).

The suggested trend towards more generalized sarcoidosis among the persons without demonstrable phage neutralizing activity may in turn be compared to the relation between generalization of bacterial infections and insensitivity to the thermo-labile bactericidal serum system of the causative bacteria, *e.g.* sepsis in man and animal, caused by seroresistant gram-negative enteric bacteria (13 9 17 16). The aforementioned inability of newborn children to neutralize phages during the first 14 days of life and their well-known sensitivity to certain septic infections during that period may also be considered.

Summary

The ability of fresh sarcoidosis sera to neutralize coli T 2 phages was examined as an indication of the natural antibody activity.

In 20 out of 90 cases tested, the T 2 neutralizing activity was lacking in contrast to the conditions in healthy persons and a number of various diseases where this activity is regularly demonstrated.

The variation of the T 2 neutralizing titers could not be significantly correlated to any special stage of sarcoidotic activity. In this material, however lacking T 2 neutralizing potency was more common among cases of generalized sarcoidosis.

References

1 Finkelstein R. A. Allen, R and Sulkin, S E Inhibition of herpes simplex virus by normal serum its relationship to the properdin system. J Infect. Dis. *104* 184 1959
2 Ginsberg H S and Wedgwood, R. J: Properdin and the inactivation of viruses. Ann New York Acad. Sc. *83* 528, 1960.
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5. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. 1. Kinetics of the coli T2 phage inactivation at various serum concentrations. Acta path. et microbiol. Scand. *51* 382, 1961
6. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. 6. Comparative studies on various phages and on sera of human and animal origin. Acta path. et microbiol. Scand, *52* 273, 1961.
7. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. Stockholm 1961
8. Kallings, L. O. and Shaw, A. The inactivation of bacterial viruses by foetal and infant sera. Acta path. et microbiol. Scand. Suppl. *154* 339, 1962.
9. Lowbury, E. J. L. and Ricketts, C. R. Properdin and the defence of burns against infection. J. Hyg. (Lond.) *55* 266, 1957
10. Lofgren, S. Snellman, B. and Nordenstam, H. Foreign-Body Granulomas and Sarcoidosis. Acta chir. Scand. *108* 405, 1955.
11. Lofgren, S. Snellman, B. and Lindgren, A. G. H. Renal Complications in Sarcoidosis. Acta med. Scand. *159* 295, 1957
12. Lundbeck, H. and Lofgren, S. Serologische Untersuchungen in Fällen von Sarkoidose. Wien. Med. Wochenschr. 1952 .0.
13. Maaløe, O. Some aspects of the normal, antibacterial defence. Acta path. microbiol. Scand. *25* 257 1948.
14. Muschel, L. H. and Toussaint, A. J. Studies on the bacteriophage neutralizing activity of serum. II. Comparison of normal and immune phage neutralizing antibodies. J. Immunol. *89* 35, 1962.
15. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W. and Wardlaw, A. C. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science, *120* 279 1954
16. Roantree, R. J. and Rantz, L. A. A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. J. Clin. Invest. *39* 72, 1960.
17. Rowley, D. The virulence of strains of bacterium coli for mice. Brit. J. Exp. Path. *35* 528, 1954
18. Scadding, J. G. Skin Reactions of Delayed Type. Proc. 3rd Internat. Conf. on Sarcoidosis. Acta med. Scand. Suppl. *425* 191 1964.
19. Skarnes, R. C. and Watson, D. W. Antimicrobial factors of normal tissues and fluids. Bact. Rev. *21* 273, 1957
20. Wardlaw, A. C. and Pillemer, L. The properdin system and immunity. V. The bactericidal activity of the properdin system. J. Exptl. Med. *103* 553, 1956.
21. Wedgwood, R. J. Ginsberg, H. S. and Pillemer, L. The properdin system and immunity. VI. The inactivation of Newcastle disease virus by the properdin system. J. Exper. Med. *104* 707 1956
22. Van Vunakis, Helen, Barlow, J. L. and Levine, L. Neutralization of bacteriophage by the properdin system. Proc. Natl. Acad. Sci. U. S. *42* 391 1956.

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4 Hinz, C. F J Jordan, W S. and Pillemer L. The properdin system and immunity IV The hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria. J Clin. Invest. *35* 453, 1956.

5. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. 1. Kinetics of the coli T2 phage inactivation at various serum concentrations. Acta path. et microbiol. Scand. 51: 389, 1961.
6. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. 6. Comparative studies on various phages and on sera of human and animal origin. Acta path. et microbiol. Scand. 52: 273, 1961.
7. Kallings, L. O. Studies on the inactivation of bacterial viruses by normal human serum. Stockholm 1961.
8. Kallings, L. O. and Alvin, A. The inactivation of bacterial viruses by foetal and infant sera. Acta path. et microbiol. Scand. Suppl. 154: 339, 1962.
9. Lowbury, E. J. L. and Ricketts, C. R. Properdin and the defence of burns against infection. J. Hyg. (Lond.) 55: 266, 1957.
10. Löfgren, S., Snellman, B. and Nordenstam, H. Foreign-Body Granulomas and Sarcoidosis. Acta chir. Scand. 108: 405, 1955.
11. Löfgren, S., Snellman, B. and Lindgren, A. G. H. Renal Complications in Sarcoidosis. Acta med. Scand. 159: 295, 1957.
12. Lundbeck, H. and Löfgren, S. Serologische Untersuchungen an Fällen von Sarkoidose. Wien. Med. Wochenschr. 1952, 40.
13. Maaløe, O. Some aspects of the normal, antibacterial defence. Acta path. microbiol. Scand. 25: 237, 1948.
14. Muschel, L. H. and Toussaint, A. J. Studies on the bacteriophage neutralizing activity of serum. II. Comparison of normal and immune phage neutralizing antibodies. J. Immunol. 89: 35, 1962.
15. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W. and Wardlaw, A. C. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science. 120: 279, 1954.
16. Rowntree, R. J. and Rantz, L. A.: A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. J. Clin. Invest. 39: 72, 1960.
17. Rowley, D. The virulence of strains of bacterium coli for mice. Brit. J. Exp. Path. 35: 528, 1954.
18. Scadding, J. G. Skin Reactions of Delayed Type. Proc. 3rd Internat. Conf. on Sarcoidosis. Acta med. Scand. Suppl. 425: 191, 1964.
19. Skarnes, R. C. and Watson, D. W. Antimicrobial factors of normal tissues and fluids. Bact. Rev. 21: 273, 1957.
20. Wardlaw, A. C. and Pillemer, L. The properdin system and immunity. V. The bactericidal activity of the properdin system. J. Exptl. Med. 103: 553, 1956.
21. Wedgwood, R. J., Ginsberg, H. S. and Pillemer, L. The properdin system and immunity. VI. The inactivation of Newcastle disease virus by the properdin system. J. Exper. Med. 104: 707, 1956.
22. Van Vunakis, Helen, Barlow, J. L. and Levine, L. Neutralization of bacteriophage by the properdin system. Proc. Natl. Acad. Sci. U. S. 42: 391, 1956.

From the Department of Internal Medicine, Lasarettet, Lund, Sweden

Lymphocyte Test in Sarcoidosis

L. Brandt R. Bouveng and A. Nordén

In March 1963 Pearmain Lycette and Fitzgerald in New Zealand published some interesting observations on the effect of tuberculin on growing lymphocytes in vitro (Lancet I 637 1963) Mitoses were induced in lymphocytes from Mantoux positive subjects without clinical tuberculosis and in cells from persons with healed tuberculosis. No mitoses were found when the lymphocytes originated from cases of active tuberculosis or from Mantoux negative subjects who had received BCG and had failed to convert. Lymphocytes were again studied in four negative cases 6 weeks after BCG immunization — in two mitoses could now be recorded.

Pearmain et al concluded that the ability of immunologically competent cells to respond to antigen by mitosis may be a general character of the immune response. Lycette & Pearmain have recently (Lancet II 386, 1963) been able to demonstrate this further by inducing mitoses in the lymphocytes from cases of hay fever and from persons immunised with poliomyelitis antigen when grass pollen extract and Sabin poliomyelitis vaccin were added to the cultures respectively

It appeared therefore of interest to apply similar techniques to the lymphocytes from cases of sarcoidosis. In a rough pilot study blood was obtained from ten blood donors. In the presence of tuberculin, without the addition of phytohaemagglutinin which is otherwise necessary the lymphocytes produced mitoses even though the number was not as great as with purified phytohaemagglutinin prepared according to Börjeson et al. (Biochim. Biophys. Acta, in press) The blood samples were delivered to us from the hospital blood bank without the names of the donors and we could therefore not check the tuberculin reaction. It could only be assumed that it originated from healthy tuberculin positive individuals as BCG immunization has been regularly practiced in Sweden for a long time on all negative reactors. We have now a more proper control series in progress, those so far reacting to 0 05 mg of tuberculin have produced mitoses in agreement with the observations reported by Pearmain et al. (1963)

Eleven cases of proven or suspected sarcoidosis out of Dr Ingestad's large series have been studied Diagnostic biopsies had been obtained from all. 30 ml of heparinized blood was collected. The lymphocytes were concentrated by passage of the blood through a glass wool column according to the technique described by Brandt et al. (Acta Med. Scandinav *172* 459 1962) nd cultured according to the method of Moorhead et al. (Exp Cell Res. *20* 613 1960) As controls, cultures with added purified phytohaemagglutinin were used and only results from cell cultures giving a high frequency of mitoses with phytohaemagglutinin have been considered

Tuberculin was added in concentrations from 625 IU to 5 000 IU per 10 ml of medium. The cultures were harvested after 72 hours, squash preparations and smears were made.

TABLE I. Tuberculin induced mitoses in lymphocyte cultures

Diagnosis (Histology)	Tuberculin Skin Test 0.05 Mg	Tuberculin Skin Test 1.0 Mg	Mitoses	Cytology typical
1. Sarcoidosis	—	—	+ (20)	?
2. Sarcoidosis	—	—	—	+
3. Sarcoidosis	—	—	—	+
4. Sarcoidosis	—	—	— (1)	?
5. Sarcoidosis?	—	+	—	+
6. Sarcoidosis?	—	—	—	+
7. Sarcoidosis	—	+	— (2—5)	?
8. Sarcoidosis?	—	—	—	?
9. Sarcoidosis?	—	+	—	?
10. Sarcoidosis	—	—	— (1)	+
11. Sarcoidosis	+		— (1)	?
12. Healthy non-BCG responding male	—	—	—	+
13. 10 blood donors	?		+	
14. 2 non-tuberculous subjects	+		+	

A 25 50 mm wide area of each preparation was examined for mitoses using magnification of 640. The smears were stained with May-Grunewald-Giemsa and attempts to make also cytological evaluations have been made. W do not yet feel that this part of the study is on safe ground and will therefore primarily refer to the presence of mitoses.

The results are summarized in table I. In the group of eleven cases suspected to represent cases of sarcoidosis, ten were found to produce no or ery few mitoses. The eleventh patient (No. 1 T ble I) was 18-year-old girl who had suffered from malaise with fever and had show an increased ESR since more than one year. Laboratory tests had suggested liver disease and biopsy had been performed which showed granulomatous changes compatible with diagnosis of sarcoidosis. She had had no pulmonary involvement and no lymph node enlargement. So far we hav no satisfactory diagnosis and do not feel that sarcoidosis is complete answer. Judging from the clinical picture it thus appears logical that she should differ from the others in the lymphocyte test. Cases number 5 and 6 showed histories and clinical pictures compatible with diagnosis of sarcoidosis but the histological pictures were not to the pathologist's satisfaction. Case number 9 occurred in 24-year-old man earlier known to giv positiv reaction to 0.05 mg of tuberculin. The day after receiving an immunization injection against poliomyelitis, he became febrile and four day later developed tender nodes on his legs. He was found to hav bilateral hilar lymph node enlargements. H no longer reacted to 0.05 mg of tuberculin but ga posit reaction to 1 mg. A scalene node biopsy showed no pathological changes.

Case number 12 in the table represents healthy student who had been given BCG on three different occasions without converting to positive tuberculin reaction. He produced no mitoses and thus beha ed as the series of cases with sarcoidosis.

Our limited experience does not permit any conclusions but the results ppear to justify further studies.

From the Sarcoidosis and Arthritis Clinics of Jefferson Medical College, Philadelphia, Pa

Latex Fixation Tests in Sarcoidosis

Harold L. Israel, John R Patterson and Nathan M Shukler

Elevated latex-fixation titers have been observed in sarcoidosis (1 2) as in other disorders accompanied by raised serum gamma globulin levels. The rheumatoid factor demonstrated by agglutination of latex particles coated with 7 S gamma globulin consists of *19 S* macroglobulins which are in effect anti-gamma globulin antibodies.

The latex fixation test appeared to deserve further study in sarcoidosis because of conflicting evidence regarding circulating antibody formation in sarcoidosis, and because of recent observations concerning the frequency of elevated titers in other pulmonary diseases (3 4)

Material and Methods

Tests were performed in 49 Negro and two white patients with acute and chronic forms of sarcoidosis attending an out patient clinic In each case the diagnosis of sarcoidosis was supported by biopsy evidence of epithelioid granuloma. The tube-dilution latex fixation tests were done by the method of Singer and Plotz (5)

Results

Agglutination ranging in titer from 1 80 to 1 5 120 was observed in 24 patients, a *frequency of 47 per cent* (Table I)

Positive tests occurred in 3 or 19 per cent of 16 males, and in 21 or 60 per cent of 35 females. Table II also indicates that reactions were more frequent in older patients.

Patients having *hilar adenopathy without* pulmonary infiltration, for the most part representing an early stage of sarcoidosis, had positive tests slightly less often than patients whose lungs had become involved (Table III)

Table I Latex fixation tests in sarcoidosis

Titer	Males	Females	Associated disease
1 5 120	—		
1 2,560	—	2	
1 1,280	1	4	Psoriasis (1 male)
1 640	—	1	
1 320	—	4	
1 160	1	2	Latent syphilis (1 female)
1 : 80	1	6	Asthma (1 female)

Table II Latex fixation tests in sarcoidosis relation to age and sex

	Number of patients	Number positive
Males, under 30	7	0
Males, over 30	9	3
Females, under 30	14	6
Females, over 30	21	15

Table III Latex fixations test in sarcoidosis relation to stage of disease

	Number of patients	Number positive
Males		
Hilar adenopathy alone	2	0
Pulmonary involvement	14	3
Females		
Hilar adenopathy alone	8	4
Pulmonary involvement	27	17

TABLE IV Latex fixation tests in sarcoidosis relation to duration of disease

	Number of patients	Number positive
Males		
Less than 1 year	2	0
More than 1 year	14	3
Females		
Less than 1 year	9	5
More than 1 year	26	16

Positive tests occurred with approximately equal frequency among patients whose disease appeared to be recent and those whose disease was known to be of more than year's duration (Table IV).

Fractionation of serum proteins by paper electrophoresis showed gamma globulin levels of 2.0 gms. or more in 33.3 per cent of the males and 41.6 per cent of the females.

Discussion

Kunkel and his associates (1) found reactions to the latex-fixation test in 6 of 61 patients with sarcoidosis, in one instance in extremely high titer. Four of the six patients had high serum gamma globulin levels, but two did not, and one patient with markedly raised gamma globulin concentration had negative latex-fixation test. They noted that all six patients with elevated titers were females with chronic sarcoidosis. None of the patients with positive latex-fixation tests showed agglutination with tanned sheep cell erythrocytes.

Positive latex fixation tests were found in 18.4 per cent of 244 patients with sarcoidosis studied by Muller Wurm and Franz (2). Elevated titres were observed in six of nine teen patients with chronic sarcoidosis.

The greater frequency of positive tests observed in our clinic is presumably the result of differences in sex and race distribution of the patients and in duration of their disease.

Impairment of delayed hypersensitivity reactions is characteristic feature of sarcoidosis, but circulating antibody formation is not correspondingly impaired. Patients with sarcoidosis have normal concentrations of tuberculosis (6) and mumps (7) antibodies and normal responses to typhoid and pertussis immunization have been demonstrated (8).

Increased circulating antibody formation has been demonstrated by Sands and his associates (9) in response to mismatched blood, and elevated antibody titers to an antigen derived from moldy hay has been found by Pepys et al. (10) in sarcoidosis patients with no history of exposure to this substance. On the other hand, impaired formation of antibodies against mycobacteriophages has been observed by Mankiewicz (11) and a diminished response to primary immunization with tetanus toxoid has been reported by Greenwood and his associates (12).

The frequency with which anti-gamma globulin antibody is demonstrable indicates that this type of antibody formation is intact in sarcoidosis. The fact that in females latex fixation reactions occurred with greater frequency than hypergammaglobulinemia, suggests an excessive immunologic response.

The course of sarcoidosis does not differ significantly in the two sexes, although the prevalence of sarcoidosis is greater in females. A sex difference in occurrence of latex fixation reactions has not been observed in patients with rheumatoid arthritis, or described in patients with hepatic disease. The greater frequency of elevated anti-gamma globulin titers in females with sarcoidosis may reflect constitutional factor in the pathogenesis of this disease.

Increased serum gamma globulin levels have long been recognized as common feature of sarcoidosis. Sunderman and Sunderman (13) and Levitt (14) reported increased gamma globulin in most patients with sarcoidosis. It is probable that these studies were performed on patients with chronic advanced sarcoidosis. Norberg (15) has recently reported comprehensive studies which include patients in various stages of sarcoidosis. Patients with early sarcoidosis as manifested by erythema nodosum had normal total protein and gamma globulin levels. In patients with hilar adenopathy and pulmonary infiltration

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Positive tests occurred in 3 or 19 per cent of 16 males, and in 21 or 60 per cent of 35 females. Table II also indicates that reactions were more frequent in older patients.

Patients having hilar adenopathy without pulmonary infiltration, for the most part representing an early stage of sarcoidosis, had positive tests slightly less often than patients whose lungs had become involved (Table III).

Table I. Latex fixation tests in sarcoidosis

Titer	Males	Females	Associated disease
1:5,120	—	2	
1:2,560	—		
1:1,280	1	4	Psoriasis (1 male)
1:640	—	1	
1:320	—	4	
1:160	1	2	Latent syphilis (1 female)
1:80	1	6	Asthma (1 female)

Table II. Latex fixation tests in sarcoidosis: relation to age and sex

	Number of patients	Number positive
Males, under 30	7	0
Males, over 30	9	3
Females, under 30	14	6
Females, over 30	21	15

Table III. Latex fixation test in sarcoidosis: relation to stage of disease

	Number of patients	Number positive
Males		
Hilar adenopathy alone	2	0
Pulmonary involvement	14	3
Females		
Hilar adenopathy alone	8	4
Pulmonary involvement	27	17

From Gustaf V:s Forskningsinstitut, Karolinska sjukhuset, Stockholm, and St. Gorans sjukhus, Stockholm, Sweden.

Serum Proteins and Glucoproteins in Sarcoidosis

RENÉE NORBERG

I will report on an investigation about proteins and glucoproteins in sarcoidosis.

The material consists of 97 patients with sarcoidosis of the hilar lymph nodes or/and the pulmonary parenchyma.

In all cases the diagnosis was supported by biopsy from lymph node. In addition to the routine examination special care was taken in liver and kidney function.

The material was divided into different groups according to the three stages of the intrathoracic sarcoidosis. I each group those patients who showed signs of current progress either roentgenologically or clinically were placed in subgroup. In this classification the author did not take part.

The BHL-group consisted of 26 patients, the group of cases with disseminated parenchymal lesions of 34 and the fibrosis group of 22 patients. The group of patients with concomitant erythema nodosum consisted of 15 cases.

Fig. 1 shows the alues of total protein and albumin in the different sarcoidosis groups. The dotted line indicates normal mean alues and the shaded area normal ranges. The dark lines indicate mean alues of the different groups. Progressiv cases are indicated by dark symbols.

The mean values of total protein are significantly elevated in all groups. As it is seen, there are mainly the progressive cases which have pathological alues. This increase of the total protein value which is mentioned by Salvesen as early as 1935, is also stated by most other investigators into this problem. As rule it has been assumed that the increase is dependent upon an actual increase of serum globulins. This also, as I shall show takes place in many cases, but it cannot be the whole truth as many patients with elevated total proteins hav quite normal electrophoresis. The dependence of the total protein alue on the posture is well-known and as most of my patients were out-patients I hav not ordinarily been able to take the blood samples from the patients before they got up in the morning. Hence I ha investigated the relation of the total protein alue to posture and ha found that the increase, which occurs when person is in supine posi-

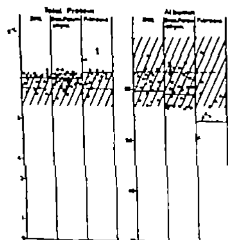


Fig. 1

representing subacute phases of sarcoidosis, total proteins were slightly but not significantly increased and gammaglobulins were essentially normal. It was only in patients with chronic forms of sarcoidosis that elevation of total protein and marked elevation of gamma globulin was observed.

These findings are consistent with the elevated latex titers noted in chronic sarcoidosis but leave unexplained the high titers occasionally observed in early sarcoidosis.

Recently attention has been called to the relationship of rheumatoid factor and pulmonary disease. Positive latex fixation tests were found in 13 per cent of 245 patients with pulmonary tuberculosis (3). Tomasi, Fudenberg and Finby (4) secured chest roentgenograms of 14 rheumatoid arthritis patients with high titres of rheumatoid factor and found that nine had pulmonary disease. Rheumatoid factor was measured in 18 patients with pulmonary fibrosis and no history of arthritis: eleven had elevated titres. Since rheumatoid factor is produced in lymph nodes, subcutaneous nodules and the synovia, and passes through the lungs, these authors suggest that antigen-antibody complexes of a certain particle size precipitate in the lungs, and result in pulmonary disease.

The hypothesis that pulmonary lesions are due to trapping in pulmonary capillaries of antigen-antibody complexes is applicable to the pathogenesis of sarcoidosis. This hypothesis accords nicely with the observation that mediastinal adenopathy invariably precedes the pulmonary infiltration, which in early stages will quickly vanish on administration of adrenal steroids. A source of large amounts of antibody is provided by the massive mediastinal lymph node enlargement often seen in early sarcoidosis. The experimental production of granulomas with antigen-antibody complexes has been reported by Germuth and his associates (16).

A demonstration of high titres of anti-gamma globulin antibody in the sera of patients with early sarcoidosis, particularly those developing pulmonary infiltration, would have provided support for this hypothesis. However this retrospective survey failed to show a clear correlation of latex fixation tests with stage of the disease. A prospective study with serial measurements on fresh cases should demonstrate whether the course of sarcoidosis is related to antibody titers.

Summary

A survey of 51 patients with sarcoidosis demonstrated positive latex fixation tests in 60 per cent of females and in 19 per cent of males. Little correlation with serum gamma globulin levels or stage of the disease was evident. The greater frequency among females appears to represent an excessive immunologic response and may reflect a constitutional factor in the pathogenesis of sarcoidosis.

References

1. Kunkel, H. G., Simon, H. J. and Fudenberg H. Arthr. and Rheumat. *1*: 289 1958.
2. Müller, W., Wurm, K. and Franz, G. Beitr. Klin. Tuberk. *462*: 470, 1961.
3. Singer, J. M., Plotz, C. M., Peralta, F. M. and Lyons, H. C. Ann. Int. Med. *56*: 549 1962.
4. Tomasi, T. B., Fudenberg, H. H. and Finby, N. Am. J. Med. *33*: 243 1962.
5. Singer, J. M. and Plotz, C. M. Am. J. Med. *21*: 888, 1956.
6. Carnes, W. H. and Raffel, S. A. Bul. J. Hopk. Hosp. *85*: 204 1949.
7. Quinn, E. L., Bunch, D. C. and Yagle, E. M. J. Invest. Derm. *24*: 595 1955.
8. Sones, M. and Israel, H. L. Ann. Int. Med. *40*: 260 1954.
9. Sands, J. H., Palmer, P. P., Mayock, R. L. and Creger, W. P. Am. J. Med. *19*: 401 1955.
10. Pepys, J., Riddell, R. W., Citron, K. M. and Clayton, Y. M. Thorax *17*: 366, 1962.
11. Makarewicz, E. Canad. M. Ass. J. *88*: 593 1963.
12. Greenwood, R., Smellie, H., Barr, M. and Cunliffe, A. C. Brit. Med. J. *1*: 1388 1958.
13. Sunderman, F. W. W. and Sunderman, F. W. J. Clin. Path. *27*: 27 1957.
14. Levitt, N. Dis. Chest. 36: 243, 1959.
15. Norberg, R. Act. Med. Scand.
16. Germuth, F. G. Jr. Am. Rev. Resp. Dis. *84*: 84 (Number 5 part 2) 1961.

normal in the BHL-group, in the other groups there are mainly the cases with signs of progress who ha e elevated gammaglobulins. The stationary cases ha mostly normal alues. As high gammaglobulins besides in subacute cases, are seen mainly in connection with fibrous-producti processes such as hepatic cirrhosis and some collagenoses, special interest has been to inv estigate the liver func tion. I hav not been able to correlate the gammaglobulin values obtained with disturbances in liv er function as it occurs in laboratory tests or as positi liver biopsies. On the other hand there is real correlation be tween gammaglobulins and pulmonary fibrosis going on. However normal gammaglobulins do not exclude the presence of ad anced fibrotic lesions, but they could then be considered to be at stationary stage.

In cases with sarcoidosis and concomitant erythema nodosum the alues hav the same tendency but more pronounced as in other progressi e sarcoidosis cases. That is de creased albumin, very high alpha$_2$-globulins, elevated betaglobulins and normal gammaglobulin alues.

The glucoprotein values are mainly cor related with the alpha$_2$-globulin values. I found elevated glucoproteins, estimated as hexoses, hexosamines, sialic acids and seromucoid in cases which clinically and roent genologically were estimated as progressi e. The highest glucoprotein values were found among the cases with concomitant erythema nodosum.

There was no evidence of glucoprotein synthesis in the sarcoid tissue, but there seems to be correlation between the occurrence of PAS-positi substance in the tissue and the glucoprotein level in serum

There is good correlation between clinical and roentgenological activity the aplha$_2$ globulin values, the glucoproteins, ESR, and C-reacti protein.

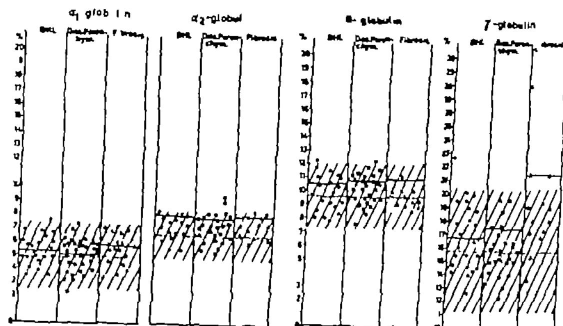


Fig. 2

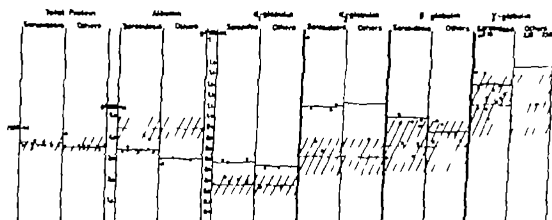


Fig. 3

tion in relation to lying is statistically higher in the sarcoidosis cases than in controls. So one of the causes of the elevated serum protein values observed could be due to this marked variation dependent on the posture. This in is turn may perhaps be connected with the pronounced orthostatic reaction frequently observed in sarcoidosis patients.

The albumin values in relativ per cent are decreased in all groups, the progressi e cases have the lowest values.

Alpha -globulins are normal.

Alpha$_2$*-globulins* are mostly normal in stationary cases and elevated in the progressi e

In many cases there is an increase of beta globulins. In the early cases this increase, *sometimes pronounced, is not accompanied* with hyperlipemia or signs of disturbances in the liver and kidney function. In older cases there is usually a decreased kidney function and signs of hyperlipemia.

The values of gamma globulins are mainly

TABLE II. Bromsulfalein retention test in cases of sarcoidosis

Group	Number examined	Pathological > 10 per cent at 30 min.	Liver biopsy	
			Number examined	Typical sarcoidosis
I	19	4	4	0
II	21	11	14	3
III	26	7	12	3
Total	66	22	30	6

traditional changes in the albumins and globulin fractions may be ery slight or absent.

The liver function has been studied by the bromsulfalein retention test in 66 of the patients (Table II). A retention exceeding 10 per cent 30 minutes after the injection of 5 mg per kg bodyweight was observed in 22 cases out of 66 studied. In the 21 cases belonging to stage II 11 showed a pathological bromsulfalein test.

Granulomatous changes in liver biopsy specimens were found only in 6 out of 30 cases. This is in contrast to the 43 per cent liver involvement reported by Mayock et al. (Am. J Med. 35, 67 1963) and 23 positive biopsies described by Sheila Sherlock in a series of 59 hepatic biopsies (S. Sherlock Diseases of the liver and biliary system, Blackwell, Oxford 1955) In autopsy material liver involvement has been reported in 60 per cent of the cases. The low figures in table II may be due, as pointed out by Dr James, to the fact that too few sections ha been studied.

DISCUSSION

Dr JAMES I agree with Professor Turiaf on the non-specificity of the Latex reaction. W did not find positive reactions in our patients when we performed the *Rose Waaler* test

W ha analysed the electrophoretic pattern of the serum proteins in 246 patients ith proven sarcoidosis. The globulins were abnormal in one-third of these patients W correlated all objective criteria and compared them in the 85 patients ith abnormal globulins and in the 161 patients with normal globulins, and to our amazement, there was virtually no differences in the two groups. The sedimentation rate is elevated, but this of course, an unimportant difference. I also seems that rises in the alpha 2 globulins ere more closely associated ith positive liver biopsies However the overall results suggest how unhelpful the serum protein levels are in assessing activity or any other facets of the disease.

Dr ISRAEL The Rose-Waaler test was done in some of our patients ith positiv latex fixation tests and was negative, as Kunkel and Wurm have reported. This does not alter the significance of the fact that the latex fixation test, representing measure of *gamma globulin-antibody* is very often positiv in sarcoidosis, especially in females. The higher frequency observed in our patients than in other studies may reflect the predominance in our clinic of Negroes who are known to have higher serum globulin levels than whites.

Dr VILLAR I analysed the 12 Portuguese cases in which the serum proteins were studied and found that in Stage I electrophoresis is normal; in Stage II (6 cases) 4 had normal electrophoresis and 2 had elevated γ globulins (some degree of fibrosis could not be excluded) In Stage III (4 cases) the albumin fraction was alway lowered and the γ globulins elevated. I one case, with an associated infection, α_2 and β globulins were also elevated. As to the sedimentation rate, it was only slightly elevated in 7 of the 19 cases studied, which is not in favour of an important dysproteinaemia.

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Serum Proteins in Sarcoidosis

R Ingestad N Tryding and Å. Nordén

Mayock et al. (Am. J Med 35. 67 1963) in their review of the records of 1,254 patients including 145 cases observed at the Hospital of the University of Pennsylvania, reported that 50 per cent of the patients had serum albumin values lower than 3.5 g per cent and serum globulins higher than 4 l g per cent, no further details were given. Hyper globulinaemia and decreased values for the albumin fraction have frequently been report ed though the degree and incidence have varied between different reports and been explained by differences in the case selection.

We ha e performed paper electrophoresis in serun from 115 patients, 50 men and 65 wom en at the Department for Pulmonary Diseases at Lund and put their material at my disposal As seen from table I the changes are not very striking Group IV has been introduced to include cases previously known to ha suffered from sarcoidosis but now apparently healed When compared to group IV the albumin fraction in the three other groups is lower and the difference has been found to be statistically significant. The α_1 and α_2 fractions are increased in groups I—III when compared to group IV β-globulins were also found to be higher in groups I—III than in group IV γ-globulins show the same increase. The differences are statistically significant in the globulin fractions when groups I—III are compared w th group IV The most marked deviations were found in stage II The normal range given in the table refers to values used in this hospital They may be less representative than the values found in group IV

The rather slight changes found in this material may be due to the fact that about half of the patients were detected at mass X-ray examinations and had at that time usually no symptoms. In the Philadelphia material described by Mayock et al. (1963) ery fe patients were without symptoms. When comparing these data with those of Dr Norberg there is no striking differences — both materials thus demonstrate that the

Table I Serum proteins n 115 cases of sarcoidosis. Median alues g per 100 ml

Group	No.	Alb.			β	γ	Total
I	36	4 77	0 27	0.54	0 72	1 11	7 5
II	40	4 45	0.32	0.62	0 76	1.34	7 6
III	36	4.63	0.28	0 57	0.74	1.35	7 7
IV	27	5.12	0.25	0.50	0.63	0.98	7 6
Normal		4.8—5.8	0.20—0.32	0.32 —0.54	0 45—0.85	0.61—1 17	6.8—8.2

in normal guinea pigs and tuberculin hypersensitive guinea pigs. The animals were sacrificed after five months. The site of injections were excised and histologic studies of the skin and subcutaneous tissue revealed isolated epithelioid cell granulomata in the hypersensitive animals. There has been some question regarding the role of paraffin oil in inciting similar lesions although the controls in this study did not show the same findings.

Subsequent experiments in rabbits exposed to long-term inhalation of pine pollen were conducted in collaboration with Dr. William Steenken of the Trudeau Laboratory. At autopsy the lungs revealed numerous collections of phagocytes filled with fat in the alveolar spaces and focal areas of peribronchial foreign body giant cell reactions (7). We did not find epithelioid cell granulomata.

Hagerstrand and Linell (8) using saline suspensions of pine pollen, injected intramuscularly into rabbits also failed to demonstrate typical epithelioid cell granulomata. They found only small round cell infiltrates of lymphocytes around the pollen deposits and foreign-body granulomata were seen on occasion in those animals which were observed for intervals up to eight months. They did confirm our finding of acid-fastness of pine pollen but found that pollen of certain other trees and plants were also acid-fast when tested with Ziehl-Neelsen stain.

The theory that pine pollen fractions might evoke or incite epithelioid cell granulomata similar to those seen in sarcoidosis, was reinforced by the observations of Vogel and Thrash (9) who noted the development of chronic granulomatous reactions in the lungs of guinea pigs and mice which were given pine pollen intranasally under ether anesthesia. Fluorescent antibody techniques identified the antigenic portions of the pollen in the lungs while auramine staining of these tissues revealed small acid-fast particles of the pollen three months after introduction of the pollen. However intratracheal insufflation of ground pine pollen in mice reported by Brieger et al. (10) failed to produce epithelioid cell granulomata in the lungs.

More recently Lindner and his colleagues (11) demonstrated remarkable epithelioid cell granulomata in 63% of 170 rats after intrasplenic or intravenous injection of whole pine pollen or pine pollen fractions and extracts. Granulomatous lesions were found in the liver of some of the rats also. With intravenous injection, most of the lesions were found in the lungs. Controls using ragweed pollen showed no granulomatous lesions. Most recently Linder (12) has found that benzene extracts of loblolly pine produce *systemic* lesions in guinea pigs and rabbits after eight months. The studies thus far indicate that the guinea pig, rabbit, mouse, and rat, under certain conditions, are capable of responding with the formation of foreign body reactions and chronic granulomatous lesions in the lungs. Whole pine incites foreign body *reactions while lipid-containing* extracts seem to evoke granulomatous lesions. To date, none of these studies in any species has led to the production of disseminated disease which can be considered to be an experimental model of sarcoidosis.

Using several serologic systems, Janicki et al. (13) *demonstrated that antibodies against* phosphatides from pine pollen could be produced in rabbits by repeated subcutaneous injections of whole pollen suspended in incomplete adjuvant as well as by pollen extracts. They found no heterologous reactivity to human tubercle bacilli although Bernton (14) *using an aqueous extract of pine pollen,* had shown delayed type of skin reactivity in some tuberculin positive guinea pigs and humans. McCuiston (15) has described similar skin reactions in normal individuals living in heavily pine-forested areas of Florida. Parnode and Leu (16) using pine pollen antigens *suitable for* detection of precipitating antigens, were unable to find such antibodies in sera of fifteen patients with biopsy-proven sarcoidosis.

These findings are of interest and possible significance because they for the first time have demonstrated that pine pollen does indeed have antigenic properties (a fact previously denied by most allergists and dermatologists) but the relationship of these antigenic qualities to the etiology or pathogenesis of

ETIOLOGICAL ASPECTS OF SARCOIDOSIS

Moderator D GERAINT JAMES

The Relationship of Sarcoidosis to Pine Pollen

From the National Institutes of Health Bethesda, Maryland.

An Evaluation of the Possible Relationship of Pine Pollen to Sarcoidosis (A Critical Summary)

MARTIN M CUMMINGS

I have chosen for the theme of my remarks a difficult and somewhat painful assignment namely a critical evaluation of my own hypothesis, advanced in 1958, which suggested that there might be a causal relationship between hypersensitivity induced by pine pollen and the development of sarcoidosis. This hypothesis stemmed from our observations that the geographic distribution of sarcoidosis occurring among American veterans correlated well with the pine forest distribution in the United States (1) Previously Gentry et al (2) had described the rural Southeastern United States distribution of sarcoidosis occurring in predominantly Negro military personnel.

Using these epidemiologic findings as a clue to the possible etiology of the disease, a systematic study of various pine forest products (chemical and biological) was undertaken. During the course of these studies, we found that pine pollen (identified since as loblolly-pinus taeda) had acid-fast staining qualities similar to those of the tubercle bacillus. Chemical studies of the pine pollen revealed the presence of long-chain fatty acids and a substance similar to the "wax" fraction of the tubercle bacillus (3) This material, which was analyzed by spectrographic and chromatographic techniques for us by Dr Edgar Lederer in Paris, proved to be different from mycolic acid. The presence of fatty acids in pollen of five coniferous species has been reported by Ching and Ching (4) Pinus contained the highest percent of fatty acid by dry weight measurement. Gas liquid chromotography revealed the presence of linoleic, oleic palmitic, and stearic acids. They suggest that variations between species may result from differences in genetic make up, climate, and maturity Our own chromatographic studies of pollen extracts revealed the presence of diammo-pimelic acid similar to that described by Work (5) and Asselineau, Choucron, and Lederer (6) in tubercle bacilli and certain other microbial agents.

Thereafter suspensions of pine pollen in paraffin oil were injected intracutaneously

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Granuloma Formation Induced by Lipid Extracts of Pine Pollen[1]

A. Lindner, T Kutkam and F Lindner

Introduction

The epidemiological and chemical studies of Cummings suggest that loblolly pine pollen might be one or the etiological factor in production of sarcoidosis (1 2) Our aim in the present study is to 1) check the biological activity of pine pollen in experimental animals, 2) determine the chemical nature of the biologically activ substance in the pollen and 3) check on long-term results of pine pollen injection. Previously w hav report ed on Experimental Granuloma Formation with Pine Pollen (3, 4, 5) describing formation of epithelioid cell granulomas in 63 of 170 rats injected with whole or fractions of loblolly pine pollen. With histochemical staining, we found positiv staining for acid-fast, sudan black B and the periodic acid-Schiff reaction

Epithelioid cell granulomas have been produced experimentally in the skin of rabbits and guinea pigs by Refvem after immunization and subsequent intradermal injection of lipovitellin plus antibody (7)

Materials and M thods

Loblolly pine pollen (Pinus taeda) grown in Georgia, USA, was used in these experiments It was collected in 19.8 with the help of the United States Forest Service.

[1] Supported in part by grant AI-04038 from the National Institute of Allergy and Infectious Diseases, USPHS.

In our studies, loblolly pine pollen, its fractions or extracts was injected intrasplenically or intravenously into rats, guinea pigs or rabbits. The methods used for the animal experiments, fractionation of pollen, histological and histochemical procedures were previously described (4) The procedure for fraction of pine pollen is shown in Chart 1 Three different species of animals were used to determine if there were differences in the reactivity of guinea pigs, rabbits and rats to the same pollen incitant.

Results and Discussion

I. *Results of Animal Experiments.* After loblolly pine pollen injections into animals, epithelioid cell granulomas were found in spleen, liver lungs and lymph nodes in about 80 % of all animals injected. The fractions of pine pollen which produced no granulomas were the supernate fraction, the chloroform-methanol extract and the KOH treated chloroform-methanol extract. Ragweed pollen produced no epithelioid cell granulomas. Also no spontaneously occurring granulomas were found in our control group of 158 animals. Details of these esults are shown in Table I

While the chloroform-methanol extract produced no lesions, the benzene extract of loblolly pine pollen injected in a single dose of 2 mg produced granulomas in about 80 % of the animals.

I addition to lesions previously reported (4) epithelioid cell granulomas were found

5—4ʃ3ʃ4

sarcoidosis remains obscure.

The epidemiological reports which have appeared in the literature during the past five years are equally inconclusive. Studies reported in the United States confirm the rural distribution of the disease in the Southeast portion of our country (15, 17 18) Terris and his associates (19) have confirmed the relationship between incidence of sarcoidosis with pine forest distribution in Louisiana. On the other hand, reports from certain other parts of the world have failed to confirm this correlation between the concentration of patients with sarcoidosis and that of pine forests. Specifically studies in Denmark (20) Scotland (21) Switzerland (22) Japan (23) and Uruguay (24) did not show such a correlation whereas, reports from Sweden (25) and South Africa (26) have confirmed the geo-ecologic relationships.

Although it is possible that the variations in findings reported may relate to difference in varieties of pine pollen, differences in animal species studied, in population samples, and epidemiologic techniques employed I am forced to conclude that my hypothesis suggesting a possible relationship between pine pollen and sarcoidosis *thus far* has not been supported by the cumulative laboratory epidemiologic, and clinical evidence available.

References

1 Cummings, M. M. Dunner, E. Schmidt R. H., Jr and Barnwell, J B Postgraduate Medicine 9 437 1956

2 Gentry J T Nitowsky H M and Michael, M J J Clin Invest *34* 1839 1955

3 Cummings M M. and Hudgins, P C. Amer J of Med Sci. *236* 311 1958.

4. Chen, T M. and Chung K. K. Science *138*, 890, 1962.

5. Work, E. Biochem. Biophysics Acta *3*. 400, 1949

6. Asselineau, J., Choucroun, and Lederer, E. Biochem. Biophysics Acta *5*. 197 1950

7 Unpublished data.

8. Hagerstrand, I and Linell, F Acta Tuberc. et Pneum. Scand. *42* 194 1962.

9. Vogel, R. A. and Thrasher, A. M. Amer Rev of Resp. Dis. *84* 81 1962 (Part 2, Proc. of the Inter Con. on Sarcoidosis, June 1—3, 1960)

10. Breger, H., LaBelle, C. W Goddard, J W and Israel, H. L. Arch. Environ. Health *5*. 470 1962.

11 Lindner, K. Kutkam A. T., Sokatch, J R. and Hammarsten J F *1* 470, 1962.

12. Personal Communication (to be published in the Transactions of the Third International Conference on Sarcoidosis, Stockholm, September 11—14 1963)

13. Janicki, B. W Aron S. A. and Branton, H W Amer Rev of Resp. Dis. *86* 385, 1962.

14 Branton H. W Ann. Intern. Med. *51* 1356, 1959

15. McCuiston, C. F., Michael, M., Jr and Hudgins, P C.: Amer Rev of Resp. Dis. *84* 124 1962 (Part 2 Proc. of the Inter Con. on Sarcoidosis, June 1—3 1960)

Patrode, R. A. and Leu R. W: Abstracted in Amer Rev of Resp. Dis. *88*. 109 1963.

17 Cooch, J W Amer Rev of Resp. Dis. *84* 81 1962 (Part 2, Proc. of the Inter Con. on Sarcoidosis, June 1—3, 1960)

18. Gundelfinger, B. F and Britten S. A.: Amer Rev of Resp. Dis. *84* 109, 1962 (Part 2, Proc. of the Inter Con on Sarcoidosis, June 1—3, 1960)

19 Terris, M., Zinnad, M M., McGill, C. and Street E. Amer Rev of Resp. Dis. *87* 590, 1963.

20 Horwitz, O Amer Rev of Resp. Dis. *84* 135 1962 (Part 2 Proc. of the Inter Con. on Sarcoidosis, June 1—3, 1960)

1 Douglas, A. C. Amer Rev of Resp. Dis. *84* 143 1962 (Part 2, Proc. of the Inter Con. on Sarcoidosis, June 1—3 1960)

22. Uehlinger, E. A. Amer Rev of Resp. Dis. *84* 153 1962 (Part 2. Proc of the Inter Con. on Sarcoidosis, June 1—3, 1960)

23 Nobechi, K. Amer Rev of Resp. Dis. *84* 148, 1962 (Part 2 Proc of the Inter Con. on Sarcoidosis, June 1—3, 1960)

24 Purriel, P and Navarrette, E. Amer Rev of Resp. Dis. *84* 155, 1962 (Part 2 Proc of the Inter Con. on Sarcoidosis, June 1—3, 1960)

25. Wallgren S. Nord. Med. *60* 1194 1958.

26. Van Linden, B. Amer Rev of Resp. Dis. *84* 162, 1962 (Part 2 Proc. of the Inter Con. on Sarcoidosis, June 1—3 1960)

TABLE I. Results of pine pollen injections

Type of experiment	Dose mg	Ratio[a]	Epithelioid cell granulomas in
Whole pine pollen	0.05	20/30	spleen
	0.5	20/32	liver lung
Supernate fraction of pine pollen	0.03	0/15	—
	0.05	13/30	spleen liver
Insoluble fraction of pine pollen	0.3	33/46	lung
	1 75	19/21	lymph node
	1 75	6/9	spleen
$CHCl_3$—CH_3OH extract of pine pollen	0.2	0/12	—
	1.0	8/12	spleen liver
Benzene extract of pine pollen	1.0	27/34	lung lymph node
	8.0	5/5	lung[b]
KOH—CH_3OH extract of pine pollen	0.5	0/5	—
Ragweed pollen	0.5	0/12	—
Control animals	—	0/136	—

Number of animals with lesion/total number of animals
Animals rats
Guinea pigs
Rabbits

acid-fast negative granulomas, we show such lesions in lungs of rabbits found 8 months after injection of acid-fast positive pine pollen extract (Figs. 4 and 5). Also seen in Figure 5 is acid-fast positive material in blood vessels of the lung resulting from a second intravenous injection 2 days prior to the sacrifice of the rabbit. The presence in the same photomicrograph of acid-fast positive material in the blood vessel and acid-fast negative granulomas in the lung appears to be evidence of the possibility of acid-fast positive material changing to acid-fast negative although 8 months appear to be necessary for the change to occur. Perhaps this is the time needed for some enzyme mechanism to adapt in order to make such changes possible.

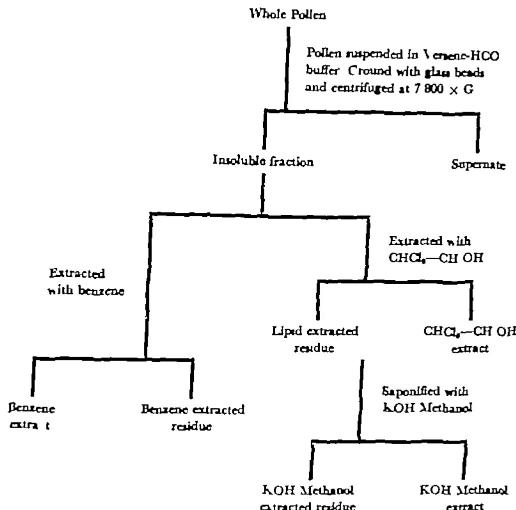
These epithelioid cell granulomas as seen after loblolly pine pollen injections are composed of loosely arranged epithelioid cells, show Langhans' giant cells but no central caseation; they appear similar to the epithelioid cell granulomas found in cases of human sarcoidosis by Uehlinger (8).

II. *Histochemical Study* of lesions with pollen fragments in giant cells showed positive staining for acid-fast, sudan black B and periodic acid-Schiff, similar to that previously reported for whole pollen (3, 4). The benzene extract also showed positive acid-fast staining (Fig. 5).

III. *Results of Chemical Analyses.* From histochemical staining reactions, lipids appeared to be associated with all lesions produced by pine pollen materials. The positive staining for acid-fast and sudan black B is suggestive of wax and the positive periodic acid-Schiff reaction for polysaccharide. Therefore, the lipids of the pollen were extracted with such solvents as chloroform-methanol and benzene. From the results of the animal experiments, it was shown that the benzene extract produced granulomas while the chloroform-methanol extract did not.

In order to identify the biologically active chemical component of the benzene extract, it was analyzed by thin-layer chromatography on silica gel with petroleum ether diethyl ether acetic acid as solvent; such chromatogram is shown in Figure 6. From this study of the benzene extract, it appears that the active substance responsible for producing the granulomas might be associated with long chain fatty acids, such as methyloleate used as standard in this chromatograph—and an alcohol, represented by octadecanol — prob-

Fractionation of pine pollen



in peribronchial lymph nodes after long-term follow-up 8 months after injection of pine pollen into the spleens of rats. Morphologically these lymph node lesions (Fig 1) are epithelioid cell granulomas similar to those previously described in spleens (4)

In these long term follow-up studies, new information on the pathogenesis of granuloma formation became available

1 Transmission of the granuloma producing material of pine pollen from one organ to another with production of systemic lesions. Eight months after injection of pine pollen, the biologically active substances have been broken down to the size of molecules, small enough to enter the blood and lymph vessels and to be carried to other organs. Liver granulomas (Fig 2) staining acid fast were found 8 months after injection of pollen material into the spleen, demonstrating this dissemination of acid-fast material from spleen to liver Epithelioid cell granulomas in lungs 8 months after injection of pine pollen into the spleen are another example of production of systemic lesions (Fig 3)

Appearance of some acid fast negative granulomas in spleens of rats 8 months after injection of acid-fast positive material. This observation was very interesting and we believe quite significant. As another example of

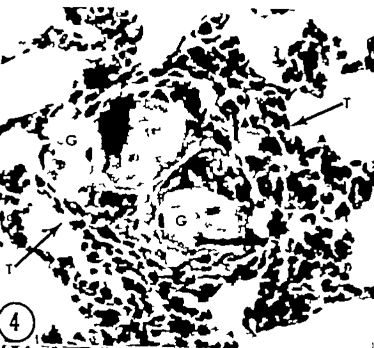


Fig. 4
Epithelioid cell granuloma in lung of rabbit eight months after intravenous injection of pine pollen. Acid-fast negative. Ziehl-Neelson stain × 500.



Fig. 5.
Epithelioid cell granuloma in lung of rabbit eight months after intravenous injection of pine pollen. Acid-fast negative. Two days prior to sacrifice of this rabbit, second intravenous injection of benzene extract of pollen was given. Acid-fast positive material from this injection can be seen in the vessel adjacent to the granuloma. Ziehl-Neelson stain .00.

Abbreviations for figures
T = tubercle
G = giant cell
B = bronchus
V = vessel





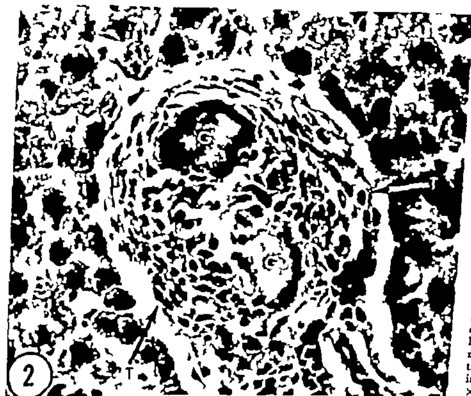


Fig. 1 *Epithelioid* cell granuloma in *peribronchial* lymph node of rat *eight months* after injection of pine pollen material into spleen. This is composed of Langhans type giant cells and epithelioid cells. H & E × 200.

Fig 2 Epithelioid cell granuloma in liver of rat eight months after injection of pine pollen into spleen. Acid-fast staining material is present in the cytoplasm of the giant cells in the lesion. Ziehl-Neelsen stain × 500.

Fig 3. Epithelioid *cell granuloma in* lung of rat eight months after injection of pine pollen into spleen. H & E × 175.

From the Department of Pathology, Allmänna sjukhuset, Malmö, Sweden.

Sarcoidosis and Pollen

Inga Hägerstrand and Folke Linell

We have not made any chemical investigations or epidemiological studies. We studied 11 kinds of pollen. Four of them were from different kinds of pines, 5 from different broad-leaved trees, 1 from grass and 1 from club-moss (Lycopodium). In contrast to earlier investigators we found all kinds of pollen to be acid-fast. Even after 24 hours treatment with acid and alcohol no kinds of pollen were completely decolorized. No appreciable difference could be found between pine pollen and other pollen in this respect.

Our experiments were done on rabbits, which we through previous studies with tuberculosis know are apt to produce epithelioid granulomas.

Earlier investigators have had difficulties in suspending pollen, because pollen is not so very wettable, and they therefore used oil as suspending medium. We succeeded to suspend the pollen in saline to such an extent that we could make the injections.

At first we injected pollen intramuscularly in the leg or intraperitoneally. The animals were studied microscopically after different periods from 0.5 to 8 months. We found clusters of pollen surrounded by foreign body granulomas. In the later stages the pollen grains were often disintegrated. No typical epithelioid cell granulomas were found.

Later on we have made a few experiments with intravenous injections of pollen, which was trapped in the lungs. We found foreign body granulomas. Many times there was rather small reaction around the pollen.

Summarizing, we have not found any typical sarcoid granulomas in our animals either locally or generalized. We found only foreign body granulomas and no difference between pine pollen and other kinds of pollen.

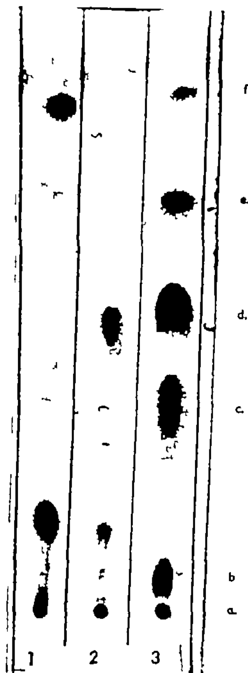


Fig 6. Thin-layer chromatogram of benzene and chloroform-methanol extracts of pine pollen. 1 benzene extract, 2 chloroform-methanol extract, 3 standard mixture of lipids a-lecithin, b-cholesterol, c-oleic acid, d-triolein, e-methyl oleate, f-cholesterol oleate.

ably in ester linkage, a waxy substance. The chromatogram of chloroform-methanol extract did not show the spots for methyl oleate (Fig 6) The study of lipid chemistry in this system is being continued.

Infrared and ultraviolet spectrophotometric analysis of the benzene extract of pine pollen was done — the dried sample was extracted with methyl alcohol and spectra prepared of both the soluble and insoluble portions. The spectrum of the insoluble material appeared to be a wax, similar to that of sugar cane wax. The soluble type material appeared to be carbohydrate. No absorption for protein in ultraviolet was noted. These tests were performed by Sadtler Research Laboratories.

Summary

Epithelioid cell granulomas were produced in about 80 of 235 animals injected with loblolly pine pollen fragments or extracts. The biologically active substance was found in the benzene extract. Chemical analysis of the benzene extract showed the presence of a mixture of a carbohydrate and a vegetable type wax.

Our findings are still preliminary while our study is continuing At present, we do not suggest that pine pollen is the cause of sarcoidosis, however it appears to have definite biological activity More details of this study are presented elsewhere (6)

References

1 Cummings, M., Dunner, E. and Williams, J H. Ann. Int. Med. 50: 879 1959.

2 Cummings, M. and Hudgins, P Am. J Med. Sc., 236. 311 1958.

3 Lindner, A., Naik, R., Kutkam, T Sokatch, J and Hammarsten, J Fed. Proc. 20 264 1961

4 Lindner, A., Kutkam, T Sokatch, J and Hammarsten, J Excerpta Med., International Congress Series, No. 41 73 1961

5 Lindner, A., Kutkam, T Sokatch, J and Hammarsten, J: Exptl. and Mol. Path. 1 470, 1962.

6. Lindner, A., Kutkam, T and Lindner, F: Z. Ges. Exp. Med. 1964. In press.

7 Reeves, O.: Acta Med. Scand. Suppl. 294 1954

8. Uehlinger, E. A. Am. Rev Resp. Dis. 84 6, 1961 Part 2.

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From the Department of Pathology University of Debrecen Debrecen, Hungary

Remarks About the Theme "The Relationship of Sarcoidosis to Pine Pollen"

J T Kelemen

Although there are no pine forests in Hungary sarcoidosis occurs in a significant number of cases and consequently we have investigated the pollen of several plants. Typha latifolia and typha angustifolia showed acid fast pollen-properties, when stained according to the Ziehl Neelsen method. Guinea pigs were treated intratracheally with the pollen of these two plants and also with the extract of their lipids. The method of examination and the results are shown in table I Details of the controls are not included.

Desoxicorticosterone-acetate/DOCA/treatment was applied to promote the connective tissue reaction. The animals were sacrificed after 7 months and examined histologically Pollens were demonstrated in the lung tissue and in the regional lymph nodes. Epithelioid cell granulation did not develop during the seven months. There was a diffuse massive interstitial inflammation in the lungs, with eosinophil leukocytes. Around the pollens or their extracts there was only a granulation consisting of macrophages and giant cells of a foreign-body type. It is our opinion that epithelioid-cell granulation cannot be provoked as a reaction to the pollen of the typha species.

Table I

	Chron. aspec. infl.	Foreign body type gran.	Epithelioid gran.
Pollen suspension	+	+	—
Pollen + DOCA	+	++	—
Lipids of pollen	+	+	—
Lipids of pollen + DOCA	+	+++	—
Controls	+	—	—

Dr Hosoda. The speaker investigated the relationship to pine pollen in 270 cases of sarcoidosis detected through the Nationwide Survey in Japan (1). Of these, 176 were histologically confirmed and the remaining 94 clinically diagnosed.

The residence of these sarcoidosis patients was rather frequently found (Fig. 1) in the areas with dense population such as Tokyo and Osaka, as previously mentioned by Dr Nobechi (2)

The pine, or pinus has more than 80 species in the world and naturally grows in the northern Hemisphere (3). According to Hayashi, there are 9 species of pinus in Japan. Fig 2 shows the geographical distribution of species of pinus commonly and abundantly found in Japan. These species are different from the foreign ones as *pinus silvestris* (Scotch pine) or *pinus taeda* (loblolly pine) *pinus elliottii* (slash pine) *pinus echinata* (short leaf pine) and *pinus strobus*. The most popular pine species in this country are *pinus densiflora* (red pine) covering the vast areas of the main islands except for the extreme northern island named Hokkaido, and *pinus Thunbergii* (black pine) growing along the coast except for Hokkaido. As shown in Fig 2, the extreme northern main island of Hokkaido is district with sparse density of pine trees. Though *pinus pumila* is relatively abundant in Hokkaido, it usually grows 800 to 2,200 meters above the sea level. In view of the fact that grows in mountainous lands and its pollen has no air bladders, the pollen of *pinus pumila* will give less influence on the inhabitants



Fig. 1 Residences of 270 cases with sarcoidosis in Japan (The Japan Sarcoidosis Research Committee)

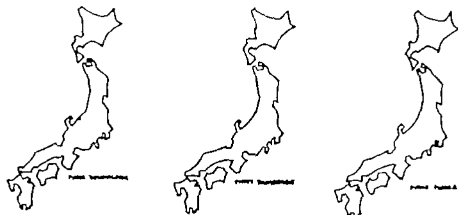


Fig. 2. Natural distribution of "Pinus" in Japan.

than the other kinds of pine pollen. In addition to these species, there are also some other kinds of pines planted for industrial purposes. For instance *pinus taeda* which was pointed out by Dunner (4) to have a close relation to the prevalence of sarcoidosis, was first planted Japan about 50 years ago. It is supposed that the inhabitants near the plantation areas of this pine have been exposed to its pollen, but th concentration of the pollen in the air would be much less than that of the domestic species. On the other hand *pinus elliottii* was just recently planted and does not yet produce pollen.

Thus, if the pine pollen is a possible cause of sarcoidosis n Japan too, there should be a difference in the incidence between Hokkaido w th sparse pine trees and the other parts of J pan with a dense pollen plantation. The results were contrary to this expectation. As shown n Fig 1 in Hokkaido there were found thirteen cases and 6 of them were born and lived there. The frequency rates for the Hokkaido population were also not very different from those in the other parts of J pan as shown in Fig I Accordingly it can be said that the residence of the cases with sarcoidosis did not afford any evidence supporting the pine pollen hypothesis as possible cause of this disease (1 5)

References

1 Hosoda, Y Proceedings of th 16th Assembly of J pan Medical Congress, III 566, 1963

2. Nobechi, K Amer Rev Resp. Dis. *81* 5 148, 1960.

3 Hayashi, Y Taxonomical and Phytogeographical Study of J panese Conifers, Norin Publishing Co. Ltd. 1960

4 Dunner, E. Amer Rev Resp. Dis. *81* 5 166 1960

5. Hosoda, Y J panese J Chest Dis. *7* 4 332 1963.

Dr Fried In 1956/57 for the first time in the Federal Republic of Germany I started an epidemiological investigation by which 1 119 cases of sarcoidosis were found in 209 West German districts. The regional distribution did *not* offer any indications that sarcoidosis might be more widespread amongst the rural than amongst the urban population. All speculations regarding any connection between the frequency of sarcoidosis and soil formation or animal husbandry also proved to be unsubstantial.

Statistics on sarcoidosis morbidity are even much less dependable than those on tuberculosis. Only one thing is certain The differences in the frequency of sarcoidosis in Germany are predominantly iatrogenous, i. e., there would be more cases, if one would look for them.

For this conference, I ha e probably gathered all the annual material on sarcoidosis in West Berlin since 1954 Especially the colleagues from screening centers and tuberculosis dispensaries knew of my particular interest and regularly supplied me with the results of their observations. Furthermore, I received material from all big hospitals.

A questionnaire, consisting of 72 items, was prepared for all patients giving me information on allergy tuberculin reaction, place of birth, exposure at work and in the family and many other questions.

First of all, I am going to deal with the *place of birth* of our sarcoidosis patients in all stages

Place of birth	Male	Female	Total
Village	10	42	52
Town	22	27	49
City	82	136	218
Total	114	205	319

Out of the 101 patients born either in villages or in towns, the overwhelming majority were resident in West Berlin for more than 5 years *before* the probable or definit beginning of the disease. Only ery few patients had ever been occupied in griculture or forestry Only one patient came from the woodworking industry another was employed in Botanical Garden. Neither was it possible to establish the fact that sarcoidosis is more frequent in the outskirts of Berlin where there are many pine forests, than in the city proper

Therefore, the animal experiments by Cummings and his colleagues can in my opinion, for the time being ha e hypothetical value only Otherwise, it would have to be proved that sarcoidosis is more frequent in certain occupational groups of forestry or woodworking industry Besides, there are regions without pine trees. Should sarcoidosis not occur there?

The Relationship of Sarcoidosis to Mycobacteria

From the Department of Internal Medicine and The William Buchanan Laboratory of the University of Texas Southwestern Medical School, Dallas, Texas

Further Studies of Mycobacterial Antibodies in the Sera of Sarcoidosis Patients[1]

JOHN S CHAPMAN and MARO SPEIGHT

Previous reports (1—6) have called attention to the presence of circulating antibodies in the serum of sarcoidosis patients. The question was raised if the reactions observed were non-specific in the sense that antigens of other mycobacteria not tested might lead to more specific reactions. The need for better geographic representation of sarcoid sera was evident and the results of studies on many more controls were desirable.

The present report deals with results of agar diffusion for mycobacterial antibodies in 280 cases of sarcoidosis, 96 of which reside outside the U.S. Antigens have been prepared from 18 additional strains of mycobacteria and employed in these tests and a considerable number of control serums from various types of patients has been subjected to study.

Materials and Methods

Diffusion in agar has been accomplished by the method previously described. In the current series, however, diffusion has been allowed to proceed for 96 hours, since it was found that the increase in time allowed for better definition of lines in the gel.

Sera of patients with sarcoidosis which have been previously reported have been restudied by this technique, as well as all current control sera.

Geographic sources of sarcoidosis sera are indicated in Table I. Control sera have come from Texas and Oklahoma with the exception of uveitis serum, very kindly supplied by Dr T F Schlaegel, Jr of the University of Indiana.

In Table II the antigens employed in tests of sarcoidosis serum are listed. Control sera were diffused only against mixed photochromogen, P 1, mixed scotochromogen, P 13, mixed non-chromogen and H37 R antigens. As will become evident diffusion of control sera against all the other antigens probably would not have resulted in significant changes in totals.

Results

In Table III the crude results of diffusion of sarcoidosis serum are presented. Only 10 sera were completely non-reactive, but 47 additional sera presented single line reactions, which are so frequent that they may be disregarded. If a positive reaction is considered

[1] This work was supported by Grant AI-04300, National Institutes of Health, U.S. Public Health Service.

Table I Geographic distribution of patients with sarcoidosis whose sera were studied

Europe		U.S.A.	
France	31	Texas — Okla.	86
England	46	Southeast	14
Sc tland	12	N Car — Va.	39
Sweden	4	West	6
Italy	1	Wisc. — Mich.	29
Canada	2	N Y — Pa.	10
	[illegible]		[illegible]

Table II Antigens employed in tests of serum

Group I	Photochromogen mixed P—1 P—8
Group II	Scotochromogen mixed P—6 P—15
Group III	Nonchromogen mixed P—2 P—17 P—39 P—44 P—50 P—54
Myco. Tbc.	H37 Rv A ian (ATCC)
Non-pathogens"	Phlei Marianum, Smegmatis, Balnei
Wild strains	Soil W ter Milk (9 Stra ns)
Fungi	2 Histoplasma Antigens 2 Blastomyces Antigens Cryptococcus Coccidioides

P — N mbers are type strains obtained from Runyon.

Table III Results of agar diffusion ag[illegible] mycobacterial antigens — sa[illegible]doids patients

	Number	Perce[illegible]
Total patients	280	100
Sera negati e	10	3.5
Sera with 1 line reaction	47	16.8
Sera considered non-reacti e	57	20.3
Sera with 2 + line eactions	223	79 7
Sera positive to H37 Rv	36	12.8

Table IV Serological reactions of control sera

Class	No.	pos. to O T 1 lin or more	pos. to un-class. 2 lines
Uveitus	98	76.3	5.3
Berylliosis	10	40	0
Fungus disease	11	0	2
Asthma	49	20	2
Medical students	11.8	27	3.4
T berculosis	250	27	32
Mycobacteriosis	61	13	49
Assorted	170	21	27
Total excluding Uveitus	669		24 7
Sarcoidosis	280	12.8	79.7

to be only a two-line or greater agar precipitin test, 223 sarcoidosis sera (79 7 %) may be considered reactive.

Table IV presents results of diffusion tests against sera from a variety of diseases. The mycobacterial patients are all those from whose sputum only unclassified acid-fast organisms have been cultured repeatedly Most of these were infections with Group I organisms similar to or identical with M. kansasii.

Other controls consist of sera from patients with asthma, with cultures positive for M. tuberculosis, with established mycotic disease of the lung with berylliosis and w th a variety of disorders such as pneumonia, emphysema with chronic bronchitis, and carcinoma of the bronchus.

Only patients with uveitis or mycobacterial infections produced reactions w th a higher than 50 frequency of that of patients with



Fig. 1 Close-up view of certain of the reactions. H37 R antigen is between 5 and 7 O'clock, and not involved in any of these reactions.

Clockwise from this point are antigens of Runyon P2, P6, and the combined antigens of Group I, of Group II and of Group III. Note the extensive cross reactions among the unclassified mycobacteria with numerous bands of identity though few more specific bands are seen. Chief reactions in the upper two systems seem to be against P2. In the lower there are certainly two, and probably more than two continuous lines from P6 around through Group I and Group II wells.

A long continuous line begins just to the left of H37 R (7 O clock) and extends all the way around to peak the Group III well. This line is frequently seen linking the antigens named, but invariably comes short of H37 R antigen.

sarcoidosis. 31 of all control sera produced two or more lines, but if the uveitis patients are removed from the group the reaction rate decreases to 24.7 *. Of the entire group of controls only uveitis patients present essentially the same serum reactivity as sarcoidosis patients.

Reactions to H37 Rv antigens, on the other hand, are about twice as common among uveitis, berylliosis and tuberculosis patients, medical students and the assorted group as among sarcoidosis patients.

Though antigens of many other strains of mycobacteria were employed only 21 sarcoid

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			1 4

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Only patients w th uveitis or mycobacterial infections produced reactions w th a higher than 50 frequency of that of patients w th

TABLE VII. Serological reactions of patients with sarcoidosis associated with erythema nodosum

Patient	Serology	Duration from E. N.
A. W. F.	S+; N− OT+ T−6+	3 Mos.
A. W. M.	S±, N±	1½ Mos.
S. W. F.	S±, N±	10 Mos.
S. W. F.	P− P−1−; S±; NC− P−6±	4 Mos.
S. W. M.	P−; S+ P−6±	2 Mos.
S. W. F.	S−; N±; P−2±; P−17±	6 Years
A. W. M.	P−; N±	5 Mos.
E. W. F.	S±; N±; P−6± P−15± P−2−; P−17−	2 Years
E. W. F.	S+; N±; OT+ P−2; P−17±	?
E. W. M.	S± N±	2 Years

the most extensive reactions of the standard antigen. In about 25 % of all cases the reaction to the various antigens was so similar that no predominance could be detected. The totals indicate that about 60 % of sera produced three or more lines of reaction, while the notes indicate the close similarity of T6 D6 to the scotochromogen antigens.

In few instances (16 patients) reactions of sera taken near the onset of disease were available for comparison with reactions as the patient began to show improvement. The results (Table VI) reveal that 12 of the 16 patients originally presented either negative or insignificant reactions which became definite and extensive as radiographic improvement occurred.

Since erythema nodosum is sometimes associated with the onset of sarcoidosis the reaction of the serum of these patients as a separate group was studied. Table VII reveals that all these patients presented extensive reactions in from 2 months to 6 years following the episode of erythema nodosum.

Discussion

If reactions to mycobacterial antigens represented general immunological hyperreactivity on an anamnestic basis one might expect to find also positive serologic tests for syphilis, high titres of agglutinins against salmonella and other similar responses. Such reactions have not been observed and antibodies to the principal fungi are not frequent in sarcoid sera.

On the other hand, at the time sarcoidosis is quite recent in onset many sera present negative or insignificant reactions, only to manifest extensive antibody response within few months as the disease regresses. (All of the patients studied sequentially thus far have been of the "benign primary" type.)

An alternative hypothesis with regard to these mycobacterial antibodies is that they are characteristic of most normal people, are depressed by the development of sarcoidosis and re-emerge as the patient achieves stability or regains his health.

It must be pointed out that the possession of antibodies against unclassified mycobacteria is not condition characteristic of sarcoidosis only. From some regions such as Oklahoma antibodies may be as prominent in controls as in sarcoidosis. At best the difference is statistical and in part is temporally dependent. But the difference is also immunological in character as well, since in these individuals without sarcoidosis but with antibodies cutaneous hypersensitivity can be demonstrated. This has been shown in medical students, in patients with uveitis, in patients with mycobacterial disease and in many of the patients with tuberculosis.

It seems unwise and unwarranted to attempt to relate the disease specifically to any particular organism. It can only be said that the greatest reactivity is directed against the antigens of P 1 P-2, P-6, P 15, and P 17 which have all been isolated from diseased human tissue. These organisms immunologically seem to be quite alike, though all three of Runyon groups are represented by these strains.

Other substances tested against some of the

TABLE V Distribution of reaction by antigens and Extent of reactions

Antigens by groups	Number of lines 2	3	4	5	6	7	Total
I	15	23	23	5	—	—	65
II	18	21	7	—	—	—	46
III	11	9	4	1	2	—	27
Mixed	31	18	4	3	—	—	56
T6 D6	9	7	7	1	—	1	25
Total	84	78	45	10	2	1	220

[1] Sera showed principal reaction to P-44

In 3 cases T6 D6 and S.C. were equal w th lines.

In .. cases T6 D6 and S.C. were equal with 3 lines.

In 1 case T6 D6 and S.C. were equal with 4 lines.

TABLE VI Cha ge in reaction from time of onset

Pt.	Locus	Original Date	Reaction	Follow-up Date	Reaction
N M.	Texas	4—59	Neg	3—60	2 line
P K.	»	9—59	Neg	6—61	2 line
C. C.	»	10—59	Neg	3—62	2 line
J L.	»	10—59	Neg	11—62	3 line
J W	»	11—59	Neg	9—61	2 line
C. L.	»	11—60	Neg.	6—61	3 line
M. S.	Fla	1—61	2 line	3—61	2 line
D P	Scotland		2 line	+ 2 Mos.	2 line
D J	Texas—J p.	9—61	Neg.	1—62	3 line
V S.	Texas	11—61	1 line	7—62	3 line
E. L.	England	9—62	2 line	2—63	3 line
V Q	England	12—62	3 line	2—63	3 line
N L.	England	12—62	1 line	2—63	3 line
R. B. B.	Texas	7—60	Neg	3—61	3 line
J T	Scotland	12—60	Neg	3—61	3 line
J G	N Mex.	4—61	1 line	5—62	3 line

sera produced reactions to these antigens that were as extensive as the reactions to the regular antigens. No significant geographic variation emerged The only wild strain antigen that produced many reactions equal to or more extensive than the type strain antigens was that indicated as T6 D6, a scotochromogen isolated from muddy soil This organism seems to be closely related to the type scotochromogens P-6 and P 15

Table V presents the distribution of reactions of sarcoidosis serum by antigen and by number of lines formed. Group I antigens elicited not only the most frequent but also

Dr Norlin After Professor Chapman's very interesting paper there are a few remarks and questions, which I would like to put forward.

As we heard, unclassified mycobacteria are not demonstrable during the early stages of sarcoidosis but are usually observed in the later stages. In addition to Professor Chapman's suggestions there may be other explanations. One explanation could, for instance, be that sarcoidosis is a precursor to mycobacterial lung infections. The few infections of this sort in Sweden have always occurred subsequent to other lung diseases, and in Holland and Belgium lung infections with unclassified mycobacteria have been detected among miners with previous lung lesions. Thus the presence of antibodies during the later stages of sarcoidosis may only indicate a secondary invasion of unclassified mycobacteria.

On the other hand let us for a moment suppose that the unclassified mycobacteria are actually an etiological agent for sarcoidosis. In that case the apparent lack of demonstrable antibodies in the early stages of the disease may be because the serum contains such an amount of antigen that the antibodies are bound. I wonder if Professor Chapman has tried to demonstrate circulating antigen by testing with immune sera. In an investigation of sera from tuberculous patients in which antibodies were not demonstrable, Parlett in Washington observed that nearly 70% of these sera contained free circulating TB antigens. Perhaps the situation is the same with the sera from the sarcoidosis patients.

I am curious about another point which concerns the interpretation of your results. You said that 25% of the patients' sera present equal reactions to antigens of the several groups and there is sufficient reaction among all antigens to suggest that the principal mycobacteria possess many antigens in common. I would like to ask you if, when you say equal reactions to antigen, you refer only to the number of precipitates formed or have you found several reactions of identity between precipitates formed in the different systems? I would also be worthwhile to know if you have found many antigenic factors common to different species.

During my investigations with a relatively large number of unclassified mycobacteria belonging to Runyon's four groups I have only found a few antigenic factors in common unless the strains belong to the same species.

The reason why I mention the question about the antigenic factors is, that with diffusion analysis according to the Ouchterlony technique the one or more species of mycobacteria which can occur with sarcoidosis might be identified.

Finally, I would like to ask why you have considered as negative those sera which form only 1 band. Have you tried to ascertain the serological character of these precipitates? The mycobacterial relation might be revealed by comparative analyses with known mycobacterial reference systems.

Dr. Nordén The delay until the antibodies may be demonstrable is not unknown in connection with other antigens. The appearance of antibodies against insulin both in man and in guinea pigs does usually not occur until three months of insulin treatment or active immunization has passed. It appears therefore not necessarily to be an argument against the serological findings in cases of sarcoidosis.

Dr Baron et Dr Vallier Les *auteurs rapportent* une observation de pneumopathie sarcoïdosique évoluant depuis plus de dix ans. Au cours d'une poussée évolutive avec augmentation et extension des images pulmonaires la culture de l'expectoration permit d'y mettre en évidence une mycobactérie atypique. Elle fut retrouvée à plusieurs reprises par culture de l'expectoration, mais sans persister au delà de quelques mois. Malgré le fait que cette mycobactérie fut retrouvée à plusieurs examens successifs, les auteurs ne lui accordent aucune importance dans la genèse de la pneumopathie, qui du reste rétrocéda par le traitement cortisonique.

Au cours des pneumopathies sarcoïdosiques la longue persistance des images pulmonaires incite à de multiples examens et cultures de l'expectoration ou des mucosités prélevées par tubage gastrique. Ces cultures peuvent permettre d'isoler des mycobactéries atypiques. Il s'agit dans la très grande majorité des cas de saprophytes provenant des voies digestives ou aériennes supérieures, et non des lésions pulmonaires elles-mêmes. *Les mycobactéries alors isolées appartiennent* aux espèces mycobacterium aquae ou mycobacterium groupe III. Au contraire, le mycobacterium kansasii *peut être responsable de pneumopathies* chroniques à évolution lente mais progressive. Ce germe dépourvu de virulence au *sens où nous l'entendons pour le cobaye*, virulent pour la souris, est responsable de lésions pulmonaires chez l'homme que il peut persister dans les lésions. On le retrouve alors à de multiples examens et pratiquement jusqu'à la mort ou l'exérèse pulmonaire. L'implantation dans les tissus *est possible en général qu'à la faveur* d'un remaniement histologique préexistant.

Sclérose pulmonaire, sarcoïdose, par le rôle qu'elles jouent sur le tissu réticulo-endothélial, amenuisant les défenses immunitaires, permettent la permanence de cette mycobactérie, sa multiplication. Elle agit alors par son pouvoir caséifiant local, transformant la pneumopathie préexistante en une pneumopathie à mycobactéries atypiques d'évolution essentiellement chronique et résistante à la plupart des antibiotiques.

more reactive sera have included *pine pollen* extract, Kveim antigen and lepramin No reactions have occurred against any of these. It seems likely therefore that if sarcoidosis is related to a living agent that agent has antigenic properties similar to those of Runyon's strains listed and to a soil scotochromogen, T6 D6. It may further be stated that many other humans have had similar antigenic contact, though in not so high a proportion of cases nor perhaps with the same degree of response.

In connection with mycobacterial infection Mankiewicz et al. (7 8) have recently reported the absence of antibodies in sarcoidosis serum against the phages of unclassified mycobacteria. Should this discovery be confirmed a possible explanation for the pathogenesis of sarcoidosis as opposed to mycobacteriosis would be unrestrained lytic activity against mycobacteria in sarcoidosis patients.

So far as the unclassified mycobacteria are concerned, their capacity to produce granuloma is well established and a few cases of bacteremia by these organisms are known Histological lesions caused by these organisms usually have been of a caseating type and from those which were not caseating it has been possible to culture organisms. Reikes and Washington (9) have produced lesions identical with sarcoidosis in mice and hamsters by injection of mycobacteria.

If there is more than a chance relationship between sarcoidosis and unclassified mycobacteria there should occur transitional clinical problems in which evidence may be confusing Such a case is family \ of Buck et al (10) a case recently seen by Dr Sol Katz (11) of Washington D C. and a similar one observed by Dr Rodger Mitchell and the senior author In all three of these the apparent diagnosis was sarcoidosis but from only one of many sarcoid lesions was an unclassified mycobacterium cultured.

Summary

1 The sera of 280 sarcoidosis patients have been diffused against antigens prepared from 24 strains of unclassified mycobacteria and against six fungal antigens. 79 7 of these sera have revealed significant reactions to mycobacterial antigens.

2 Sera from 770 control cases have been diffused in agar against the more reactive antigens and 31 % of these sera have also shown significant reactions. In Indiana patients with uveitis the reaction rate has been essentially the same as that of sarcoidosis patients. In limited areas controls have reacted in the same manner as sarcoidosis.

3 The reactions of 16 sarcoidosis sera have changed from negative or weakly positive to quite reactive in benign primary cases as radiographic clearing has occurred.

4 The reactions of sarcoidosis patients with erythema nodosum in their history is similar to that of other sarcoidosis patients.

5 Theoretical possibilities associated with these serological reactions are discussed.

References

1 Muller, W Wurm, K. and Reindell, H. Beitr zur Klin. Tuberk. *118*. 299 1958.

2 Decroix, G., Grisland, P et Kourilsky R. Journ. Franc. de Med. Thor 9. 638, 1955.

3. Muller, W Wurm, K. and Franz, G. Beitr zur Klin. T berk. *124* 462, 1961

4 Pattmode, R. A. and Lau R. W (Abst.) Amer Rev Resp. Dis. *88* 102 1963.

5 Chapman, J S. Proc. Soc. Exper Biol. and Med. *107* 321 1961

6 Chapman J S. Ann. Int. Med *55*. 918, 1961

7 Mankiewicz, E. J Gen Microbiol. *24* 63, 1961

8. Mankiewicz. E. Canad. Med. Ass. J *85*. 593 1963.

9 Reikes, H. and Washington W Amer J Clin. Path. *38*. 244 1962.

10 Buck, A. A. and McKusick, V A. Amer J Hyg *74* 174 1961

11 Katz, H. Personal communication.

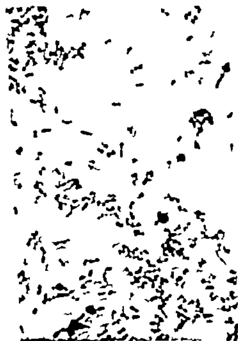


Fig. 2. Large bodies, acid-fast free granula (stained according to Ziehl-Neelsen 800)



Fig. 3. Microscopic examination of cultures after repeated subcultures on phage antisera containing media: bacillary forms emerging from large bodies (stained according to Ziehl-Neelsen ,00)

After several passages in phage anti-serum containing media, there appeared colonies which were umbilicated, pigmented, resembling those of anonymous mycobacteria (Fig. 1)

On first isolation, the microscopic examination showed large bodies, coccoid and clubbed bacillary forms, ring forms (Fig. 2) Only some free granula were acid-fast. After repeated subcultures on antiserum medium, bacillary forms emerged from the large bodies (Fig. 3) and finally the cultures showed elongated bacilli with acid-fast granula (Fig. 4)

I some instances it was possible to demonstrate the origin of these ariant strains as lysogenic mutants because they produced themselves particles lytic for other phage-sensitive mycobacteria. In other instances, the presence of pro-phage could only be assumed on the basis of the resistance of these bacteria to phagolysis.

It was then attempted to reproduce sarcoidosis in experimental animals. The inoculation of the usual infecting doses of tubercle bacilli (0.5—1 mg) together with that of mycobacteriophages gav us the same results as obtained by Hauduroy and Rosset (3) widespread tuberculosis with caseous lesions, and high degree of tuberculino-allergy. On the other hand, the inoculation of the stable mycobacterial ariant strains isolated from patients with sarcoidosis, or produced in vitro by lysogenization, created lesions which the pathologist qualified as of the chronic inflammatory type"

Remembering the anergic state of the patients with activ sarcoidosis, their failure

From the Royal Edward Laurentian Hospital and the Department of Bacteriology McGill University Montreal Canada

The Relationship of Sarcoidosis to Anonymous Bacteria[1]

EDITH MANKIEWICZ

In 1961 it was reported that bacteriophages lytic for mycobacteria can be isolated with great frequency from stool- and resection specimens from patients with tuberculosis and sarcoidosis while patients with other than those diseases are seldom found to harbour mycobacteriophages (1)

While all tuberculous patients infected with mycobacteriophages had raised high titers of phage neutralizing antibodies, patients with sarcoidosis showed no antibodies to phages lytic for only virulent tubercle bacilli, such as strain DS6A they showed none or only very low antibody titers for phages lytic for saprophytic and virulent mycobacteria (2)

In vitro exposure of phage-sensitive mycobacteria to mycobacteriophages results in the emergence of phage resistant atypical colonies. This observation led us to attempt to isolate such mycobacteria from biopsy specimens collected from patients with sarcoidosis and found to carry mycobacteriophages. From 12 specimens, after 2—3 months incubation of cultures in thioglycollate broth and in other media enriched with phage neutralizing rabbit sera, we isolated 7 strains of atypical mycobacteria.

On first isolation, the colonies of these organisms were very unlike those of *M tuberculosis* They were viscous, non pigmented



Fig 1 Colonies of typical mycobacteria from biopsy specimen from patient with sarcoidosis. HS: after several passages in horse serum containing media. AS DS6 after several passages in media containing phage neutralizing rabbit antisera.

This research was supported in part by a Medical Research Council Grant and in part by a grant from the Muskoka Memorial Research Fund.

TABLE I. Isolations of typical and of atypical mycobacteria from autopsy specimens of guinea pigs infected with tubercle bacilli or tubercle bacilli and mycobacteriophage

	Percentages of cultures of Mycobacteria isolated from total of specimens collected from guinea pigs inoculated with			
	H37R	H37R	H37R + phage D56A	H37R + phage D56A
receiving	—	cortisone	—	cortisone
Specimens from				
Ext. iliac lymph node	90	25	80 ( )	80 (d)
Liver	25	55	10 (b)	60 ( )
Spleen	60	65	35	35
Lungs	30	80	21 ( )	30 (f)

( ) one culture of 16 was composed of typical and "atypical" colonies of mycobacteria;
(b) one colony of three colonies yielded by one of the two positive cultures in this group was "atypical"
( ) three of the five positive cultures in this group showed "atypical" colonies among typical ones;
(d) 10 cultures out of 16 showed "atypical" pigmented smooth colonies
( ) three cultures out of 12 and
(f) five cultures out of six showed "atypical" colonies

TABLE II. Phage neutralizing antibodies in sera of experimental guinea pigs

	Presence of phage neutralizing antibodies in sera of guinea pigs inoculated with			
	H37R	H37R	H37R + phage D56A	H37R + phage D56A
receiving	—	cortisone	—	cortisone
Total of sera tested	15	11	16	15
Neutralization of phages				
D56A	—	—	15	3
DW	5	—	12	3
Lev	—	—	15	9

releases variant phages. This has already been shown by Froman, Jann and Russell (5). Phages of different antigenic composition determine a shift in the antibody reaction such as we have seen. Antibodies and phages act as selective agents and determine the survival, or the length of survival, of certain mycobacteria, with biological characters and pathogenicity different from that of the parent strain.



Fig 4 Microscopic examination of stable mutant beaded bacillary and filamentous forms which are acid-fast (stained according t Ziehl-Neelsen × 700)

to raise phage neutralizing antibodies, we attempted to reduce the antibody response to the infection w th bacteriophage by treating the animals for 7 weeks out of an observation period of 10 weeks with pharmacological amounts of cortisone Indeed, we had found that such treatment reduced considerably the antibody level produced in normal rabbits by vaccination w th mycobacteriophage.

At the same time we reduced the nfecting doses of tubercle bacilli strain H37R to 1 mcg per animal, and increased that of mycobacteriophage to 10 million lytic parti cles of phage DS6A. This phage is specific for virulent tubercle bacilli and has been isolated and described by Redmond and Cater (4)

After 9 weeks, all the surviving animals in the Control group infected w th only tubercle bacilli reacted to Old Tuberculin, and a few reacted to a similar skin test made with a suspension of a lysogenic variant of tubercle bacilli. Guinea pigs infected with tubercle bacilli and kept on cortisone did not react. Those infected with tubercle bacilli and mycobacteriophage were highly allergic only two of those receiving cortisone reacted to OT In both latter groups, there was a high incidence of reactors to the lysogenic variant.

Following autopsy of the experimental animals, portions of lymph nodes, spleen and lungs were cultured for typical and atypical mycobacteria. Only from the animals infected with phage, atypical mycobacteria were isolated, either alone or together with typical *M tuberculosis*. The atypical" bacteria were distinct from the typical ones by their smooth texture and pigmentation. Many produced phage particles lytic for the parent strain H37Rv for strains ATCC607 and other saprophytic strains (Table I) From the same specimens, mycobacteriophages could be isolated Most of these had a much larger lytic spectrum than phage DS6A. This phage variation was reflected also in *the shift in* phage neutralizing antibodies which were determined in the serum of the experimental animals 10 weeks after infection. The sera were tested for antibodies neutralizing the infecting phage DS6A, a mycobacteriophage which we call DW and which had been isolated from a patient with tuberculosis due to atypical mycobacteria, and against phage Leo which had been isolated from a lung biopsy from a patient with histo-pathologically confirmed sarcoidosis. The results of phag neutralization tests are tabulated in Tabl II

Five animals in the group infected with only tubercle bacilli, showed antibodies to phage DW Animals infected with tubercle bacilli and phage DS6A neutralized not only phage DS6A, but also the unrelated phages DW and Leo The administration of cortison for part of the experimental period repressed the response to the specific antigen DS6A, but 60 of the sera neutralized the mycobacteriophage that had been isolated from the patient with sarcoidosis.

These findings together w th others which we have no time to report suggest H37Rv lysogenized with on type of bacteriophage,

Summary

A high percentage of guinea pigs infected with small numbers of tubercle bacilli and large number of lytic particles of mycobacteriophage DS6A showed "sarcoid-like reactions" in lymph nodes and lungs. From these tissues, "atypical" mostly lysogenic mycobacteria were isolated. The administration of cortisone increased the number of isolations of atypical bacteria.

A theory is proposed to explain the origin of the "atypical" mycobacteria as resulting from an interplay between phages, variant phages and the corresponding shifts in antibodies. As tubercle bacilli alone and the atypical mycobacteria resulting from lysogenization and stabilized by treatment with phage antisera did not produce "sarcoid-like lesions", it must be assumed that these lesions are caused by one of the mycobacterial forms which, because of phage actions and by antibody selection, are of transitory existence.

References

1. Mankiewicz, E.: Nature 191: 1416, 1961.
2. Mankiewicz, E. and van Walbeek, M.: Arch. Environm. Health, 5: 122, 1962.
3. Hauduroy, P. et Rosset, W.: C. R. Soc. Biol. 154: 285, 1960.
4. Redmond, W. B. and Cater, J. C.: Amer. Rev. Respir. Dis. 82: 781, 1960.
5. Russell, R. L., Jann, G. J. and Froman, S.: Amer. Rev. Resp. Dis. 82: 384, 1960.

COMMENT

Dr Kallings: Dr Mankiewicz has made enthusiastic efforts in her interesting approach to the etiology of sarcoidosis, presenting results which embrace a whole series of bacteriological problems. Because of the long generation time and the special substrate needs of mycobacteria, these problems, intricate each in themselves, must furthermore be of greater difficulty than in comparable basic work which is done, for instance, with coli bacteria.

Even greater obstacles are, of course, met with when transferring from in vitro experiments to the experiments with animals reported. This is a kind of applied ecology which has not been done much in classical phage research. In the absence of more detailed information, one finds it difficult, at present, to form an opinion on the importance of the results reported and their clinical interpretations.

Thus, the finding in sarcoidosis patients of high frequency of mycobacteriophages, but no finding of neutralizing antibodies against these phages, is a matter open for discussion. To what extent do mycobacteriophages occur circumstantially or transiently in sarcoidosis patients in connection with a stay in a tuberculous infected environment or through steroid treatment, leading to the appearance of saprophytic mycobacteria harbouring phages lytic to the test strains used in the experiments?

The references concerning the technic for the neutralization tests, which Dr Mankiewicz has described, do not make completely clear if the experiments are carried out so that only the specific antibodies are measured.

In spite of the anergy there is in the literature, after all, no general support for the assumption that the production of circulating specific antibodies should be reduced in sarcoidotic patients. Of great interest would be direct experiments, where the antibody response is measured in sarcoidotic patients, who have previously received purified preparations of mycobacteriophages. It would also be of particular interest to follow during a long period, the excretion of mycobacteriophages from separate individuals with sarcoidosis, and to correlate the findings of phages with the presence or absence of neutralizing antibodies.



Fig Retro-tracheal lymph node of guinea pig infected with t bercle bacilli and mycoba teriophage *DS6A* and recei ing cortisone. Sarcoid like lesions" (stained by H. P S 18)



Fig 6. Multi-nucleated giant cells (stained by H. P S. x 450)



F'g 7 Granulomatous sarcoid-like lesions in lung of guinea pig infected with human type tubercle bacilli and mycobacteriophage DS6A. (stained by H P S. 60)

The histopathology of the lesions in the experimental animals was studied by Dr Jean Béland, pathologist of the Royal Edward Laurentian Hospital and Director of the Department of Laboratories of the Santa Cabrini Hospital This was a blind" study as he did not know to which on of the four groups of animals the specimens belonged.

In all animals infected w th only tubercle bacilli, receiving cortisone or not, the pathologist reported lesions with caseation necrosis and the presence of acid-fast bacilli.

In both groups of animals infected w th tubercle bacilli and mycobacteriophag he found only very few acid-fast bacilli, but many intra-cellular or extra-cellular purpl granula.

In the phage infected animals, lesions de scribed as granulomatous, sarcoid like" were reported in 17 out of 20 iliac and retrotracheal lymph nodes, and the same type of lesions were found in three specimens of lungs.

In the animals infected w th tubercl bacilli and mycobacteriophages and receiving cortisone for 7 weeks out of 10, 12 out of 20 ani mals showed sarcoid reactions in their lymph nodes, and 4 animals showed similar lesions in their lungs (F'g 5—7)

W evaluated 38 different tissue suspensions, 21 of our own and 17 submitted preparations, and only 18 or less than half of them were satisfactory. The other 20 were unacceptable and had to be rejected, 11 because they were too weak and 9 because they elicited granulomas nonspecifically.

For validating and calibrating the specificity and sensitivity of K eim test suspensions, *it is necessary to carry out* large number of tests in a steady flow of patients, including those with sarcoidosis, those suspected of having the disease, and equally important, subjects w are reasonably sure will provide no evidence of it.

Evolution of Kveim Reaction. By means of injections spaced one week apart for four weeks, one can follow the histologic transformation of predominantly mononuclear cell response which is present after one week to the characteristic follicular epithelioid-cell accumulations at three to six weeks. It is not clear whether the mononuclear cells with their dark-staining nuclei which ppear early giv rise to the epithelioid cells with vesicular nuclei or whether the epithelioid cells evolv from cells which replace the mononuclear cells. But it is clear that neutrophiles, eosinophiles and plasma cells, present in small numbers during the first and second weeks, diminish in the later weeks as the epithelioid-cell follicles mature. PAS-positive material, acid mucopolysaccharides, phospholipids and acid phosphatase may be found in giant cells, epithelioid cells and in areas of fibrinoid change. As in the healing of spontaneous lesions of sarcoidosis, the epithelioid-cell follicles may be surrounded and eventually replaced by hyalinized connectiv tissue in six months to year but microscopic fibrosis of the K eim site granulomas may be shortened in patient receiving corticosteroid therapy.

Fluorescent antibody stains of Kveim sites and sarcoidal lymph nodes. Fluorescen antibody studies for the presence of gammaglobulin and component of complement (C'3) were performed in collaboration with Dr F Paronetto of the Mount Sinai Hospital on 10 K eim test site specimens one to four weeks old and on fiv freshly excised sarcoidal lymph nodes. In none of these specimens could w detect abnormal amounts of gammaglobulin or of the complement component within or outside the granuloma. Fibrinogen could be detected by the immunofluorescent techniques and was located around but not within the granulomas in eight K eim sites as well as in the tw sarcoidal lymph nodes. Fibrinogen was present within the granulomas only in areas of fibrinoid necrosis. W do not yet know the significance of this finding.

Electron Micrography of Kveim test site and sarcoidal lymph node. Figure 1 depicts part of an epithelioid cell present in four-weeks-old K eim site enlarged 20 000 times. In the upper right hand corner the normally thin cleft-like *endoplasmic reticulum is preserved.* I the lower left, however the endoplasmic reticulum is dilated. Some of the endoplasmic reticulum is lined by ribosomes and there are many free ribosomes in the cytoplasm. A large



Fig. 1 Electron micrograph of epithelioid cell from four-weeks-old K eim test site 20,000. For detailed description, see text.

Nature, Significance and Interpretation of the Kveim Reaction

From the Division of Thoracic Diseases, Dept. of Medicine, The Mount Sinai Hospital, New York, New York.

Significance and Specificity of the Kveim Reaction[1]

LOUIS E. SILTZBACH

I shall begin by outlining the properties possessed by a satisfactory human sarcoidal tissue suspension or so-called Kveim suspension which is employed as a diagnostic agent in sarcoidosis.

First, the processed tissue suspension must contain a high enough concentration of the granuloma-producing factor or factors to evoke positive Kveim reactions in a majority of patients with *active* sarcoidosis- six or more of every ten subjects tested.

Second a properly screened suspension elicits in a responsive subject a histologically characteristic cutaneous papule within four to six weeks. The papule is composed of compact and discrete masses of epithelioid cells and occasional giant cells, exhibiting only a minor degree of fibrinoid necrosis and nonspecific inflammatory cellular reaction. Birefringent bodies may occasionally be present in small numbers.

Third, the papule grows slowly and in about one third of responsive subjects measures at least 5 mm in diameter after four to six weeks. The other two-thirds of the Kveim-positive subjects may display smaller papules.

The size of the papule accurately measured, is a reliable guage of the *level* of Kveim reactivity in responsive subjects. The tissue particles of a satisfactory suspension are fine and are easily dispersible by shaking the vial. Simultaneous injection of an equal volume and concentration of the suspension in a responsive subject should yield like-sized papules in four to six weeks.

Fourth, an effective Kveim suspension is bland producing in non-sarcoid subjects no sizeable papule and no sarcoidal reaction histologically. False positive reactions do not exceed a level of three per cent and, when they are present at all, they are characterized by small papules at six weeks which histologically exhibit only a few granulomas with little or no admixture of nonspecific inflammatory cells.

Tissue Source of Kveim Test Suspensions The sarcoidal tissue, preferably splenic tissue, is obtained from a patient with known sarcoidosis and the tissue should exhibit a characteristic histologic pattern. Tissues showing relatively fresh granulomas with minor degrees of hyalinization if any are preferred. The tissue should contain no detectable bacteria or fungi when stained and cultured by appropriate techniques. There should be no history of recent blood transfusion or jaundice.

[1] This study was supported by Grant AI-02272 from the National Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

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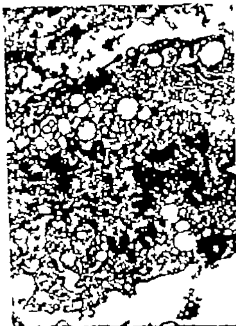


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Tissue Source of Kveim Test Suspensions. The sarcoidal tissue, preferably splenic tissue, is obtained from a patient with known sarcoidosis and the tissue should exhibit a characteristic histologic pattern. Tissues showing relatively fresh granulomas with minor degrees of hyalinization if any are preferred. The tissue should contain no detectable bacteria or fungi when stained and cultured by appropriate techniques. There should be no history of recent blood transfusion or jaundice.

[1] This study was supported by Grant AI-02272 from the N tional Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

We evaluated 38 different tissue suspensions, 21 of our own and 17 submitted preparations, and only 18 or less than half of them were satisfactory. The other 20 were unacceptable and had to be rejected, 11 because they were too weak and 9 because they elicited granulomas nonspecifically.

For validating and calibrating the specificity and sensitivity of Kveim test suspensions, it is necessary to carry out large number of tests in steady flow of patients, including those with sarcoidosis, those suspected of having the disease, and equally important, subjects w are reasonably sure will provide no evidence of t.

Evolution of Kveim Reaction. By means of injections spaced one week apart for four weeks, one can follow the histologic transformation of predominantly mononuclear cell response which is present after one week to the characteristic follicular epithelioid-cell accumulations at three to six weeks. It is not clear whether the mononuclear cells with their dark-staining nuclei which appear early give rise to the epithelioid cells w th vesicular nuclei or whether the epithelioid cells evolv from cells which replace the mononuclear cells. But it is clear that neutrophiles, eosinophiles and plasma cells, present in small numbers during the first and second weeks, diminish in the later weeks as the epithelioid-cell follicles mature. PAS-positive material, acid mucopolysaccharides, phospholipids and acid phosphatase may be found in giant cells, epithelioid cells and in areas of fibrinoid change. As in the healing of spontaneous lesions of sarcoidosis, the epithelioid-cell follicles may be surrounded and eventually replaced by hyalinized connective tissue in six months to year but microscopic fibrosis of the K eim site granulomas may be shortened in patient receiving corticosteroid therapy.

Fluorescent antibody stains of Kveim sites and sarcoidal lymph nodes. Fluorescent antibody studies for the presence of gammaglobulin and component of complement (C'3) were performed in collaboration with Dr F Paronetto of the Mount Sinai Hospital on 10 Kveim test site specimens one to four weeks old and on five freshly excised sarcoidal lymph

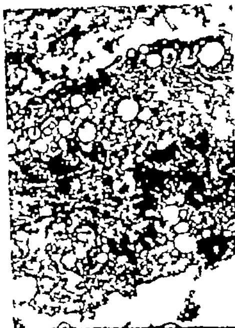


Fig. 1 Electron micrograph of epithelioid cell from four-weeks-old Kveim test site 20,000. For detailed description, see text.

nodes. In none of these specimens could w detect abnormal amounts of gammaglobulin or of the complement component within or outside the granuloma. Fibrinogen could be detected by the immunofluorescent techniques and was located around but not within the granulomas in eight Kveim sites as well as in the two sarcoidal lymph nodes. Fibrinogen was present within the granulomas only in areas of fibrinoid necrosis. We do not et know the significance of this finding.

Electron Micrography of Kveim test site and sarcoidal lymph node. Figure 1 depicts part of an epithelioid cell present in four-weeks-old Kveim site enlarged 20,000 times. In the upper right hand corner the normally thin cleft-like endoplasmic reticulum is preserved. In the lower left, however the endoplasmic reticulum is dilated. Some of the endoplasmic reticulum is lined by ribosomes and there are many free ribosomes in the cytoplasm. A large



Fig 2 Electron micrograph of epithelioid cell from sarcoidal lymph node 20,000 For detailed description see text.

number of cytoplasmic vacuoles contain phagocytized material. There is condensation of dense staining material in the periphery of the nucleus.

Figure 2 shows a section of a sarcoidal lymph node One sees part of an epithelioid cell with some of its nucleus appearing at the left There is considerable dilation of the endoplasmic reticulum Again some of the endoplasmic reticulum is lined with ribosomes. Some cytoplasmic vacuoles contain finely granular phagocytized material, commonly seen in macrophages. The mitochondria and the Golgi apparatus are not clearly seen. The overall appearance of the cell is similar to the one seen in the four weeks-old Kveim test biopsy specimen.

Not many conclusions can be drawn at present from the similarity between the epithelioid cells of the spontaneously occurring sarcoidal lesions in the lymph node and the lesions of the four weeks-old Kveim test site. More work in this area is under way in the laboratories of Dr James G Hirsch of the Rockefeller Institute.

Whatever this granuloma-evoking material may be, whether bacterial, fungal or viral residues or some unknown chemical agent of exogenous or endogenous origin, it induces a tissue reaction within the dermis which is very similar histologically and histochemically to that seen in the natural lesions of sarcoidosis (2) Non-sarcoid human tissues do not possess this capacity It may be concluded that the same unknown substance or substances which elicit granulomas characterizing sarcoidosis are present as well in the tissue suspensions and in the Kveim papules they induce.

On one occasion a Kveim suspension prepared from a freshly excised Kveim papule was reinjected into the skin of the same patient from whom it came. There developed a slowly maturing papule at the new site which, on biopsy showed the characteristic appearance of a positive Kveim reaction.

Physical and chemical treatment of Kveim suspensions. With Drs. Merrill W Chase, James G Hirsch, Zanvil A. Cohn, and Stephen I Morse of the Rockefeller Institute, studies have been undertaken to determine the nature of the granuloma-provoking substance. We have subjected our Type I suspensions (Chase-Siltzbach, 1—3) to various physical and chemical treatments (Table I)

The active principle of the Kveim suspension is remarkably stable it withstands removal of lipids by extraction with chloroform-methanol 2 1 Subsequent removal of nucleoprotein by 1*M* NaCl which further reduces the dry weight, also leaves the activity of the Type I Kveim preparation unaffected (5) Acids destroy little of the activity but the active principle is labile in 1/20 N NaOH with considerable reduction of activity even after 90 seconds of treatment. Treatment with formalin, butanol and 95 ethanol causes little alteration in activity of the suspension.

Immersing the Kveim suspension in boiling water for 30 minutes reduces the effectiveness of the suspension by half and autoclaving for 20 minutes almost completely in-

Table I *Effect of Various Chemical and Physical Treatments on the Activity of Kveim Suspensions*

	Duration of treatment	Temp. C	Effect on Kveim activity
Chloroform methanol 2 1	6 days	0°	Little altered
Above plus 2 M NaCl	4—7 hrs.	4	
Acid 0.1 N HCl	4 day	37°	Slightly reduced
1.0 N HCl	4 hrs.	100°	Moderately reduced
0.017 M HAc	36 hrs.	0	Little altered
50 TCA	½ hr.	0	
50 % TCA	½ hr	90°	
Alkali 0.03 N NaOH	24 hrs.	37	Inactivated
0.03 N NaOH	1 hr	0	Almost inactivated
0.03 N NaOH	90 sec.	0°	Markedly reduced
pH 8.3—10.5	18 hrs.	0	Little altered
pH 11.9	18 hrs.	0°	Almost inactivated
Buffered formalin	24 hrs.	4	Little altered
Ethanol 95 %	1 hr	20°	
Butanol	36 hrs.	0	
Boiling	1 hr	100°	Moderately reduced
Autoclaving	20 min.	120°	Almost inactivated

In collaboration with Drs. Merrill W Chase, James G Hirsch, Zanvil A Cohn, and Stephen I Morse.

activates it. However unheated and nonphenolized suspensions are not superior to the heated, phenolized product. Repeated freezing and thawing has no deleterious effect nor does prolonged sonication. The suspension retains activity even after passage through an ultra-fine sintered glass filter when the filtrate is brought back to original concentration (1 2) Furthermore, the test suspension can be diluted 400-fold so that only one gamma of tissue is delivered per dose; positive Kveim reactions are still obtained even in moderate reactors. Doubling or tripling the standard dose does not increase the yield of positive reactors.

Specificity of the Kveim Reaction The Kveim test performed with adequately screened tissue suspensions has proved, in our hands, to possess satisfactory specificity in the diagnosis of sarcoidosis. We have recently completed Kveim test study of 38 patients with beryllium disease, a condition which simulates sarcoidosis in many respects. This study was carried out in conjunction with Drs. H. Hardy J Stoeckle, J Lieben and J Dattoli. None of these 38 patients with beryllium disease responded to our Type I Kveim suspension. Twenty-three patients with leprosy were also Kveim-tested. Of these, two patients, both in Japan, showed weakly positive Kveim reactions with somewhat typical histological features. Thus, Type I suspension (Chase Siltzbach) appears to cause weak, unusual looking cross-reactions in the skin of patients with lepromatous leprosy in Japan but not in similar patients in some other countries; — Israel, Finland and Italy More patients with leprosy in Japan are now receiving Kveim tests with our Type I suspension and new study is being carried out with the same Kveim suspension among patients with leprosy in Korea as well.

Among 115 patients with tuberculosis, mostly in an active phase, two patients exhibited weakly positive reactions, an incidence of less than 2 false-positive tests.

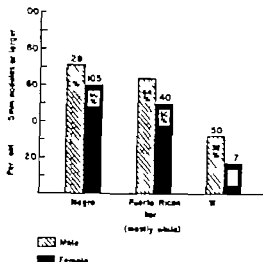


Fig 3. Specific K eim Nodules 5 mm nd larger by patient sex and race.

Factors controlling Size of Kveim Papules Figure 3 shows that whereas 71 of responsive Negro males exhibited K eim papules 5 mm and larger only 32 of white males d d so. A similar relationship existed between the women of the t o races 60 w th large papules among the Negro women and 17 among the white women. Puerto-Rican-born subjects, mostly white also exhibited good sized K eim papules. In all three groups, the men showed stronger Kveim eactions than did the women. The degree of tuberculin sensitiv ty did not appear to have any effect on the size of the K eim papule

Results f Kveim Tests at The Mount Sinai Hospital Since 1946 we ha e tested 1 013 patients w th our K eim suspensions and these included 417 patients who where diagnosed as having sarcoidosis. Among 219 patients with biopsy-confirmed sarcoidosis, th K eim tests were positi e in 85 % of instances, whereas among suspects the positive reactions amounted to 55 Among 350 patients *without* evidence of sarcoidosis there were two false positive reactions, an overall incidence of false positive reactions of less than 1 %. Tests were repeated at various intervals in 108 Kveim-reactive patients. One year after the first positive Kveim test, 19 had lost their responsiveness. Loss of Kveim reactivity increased to 30 after one to three years and in three to five years, only 47 % of the total were still positive. At the end of five to 15 years, the incidence fell to 33 It is clear that as the disease recedes or grows more chronic, the level of Kveim reactivity falls appreciably

In summary the K eim test when properly carried out with suitable test material is a valuable aid in the diagnosis of sarcoidosis. Its specificity extends beyond that associated with the characteristic changes visible in an organ biopsy specimen. W thout minimizing the value of organ biopsy one s feeling of confidence in the diagnosis rises considerably when a positive Kveim reaction is elicited in any patient being investigated for possible sarcoidosis.

We regard Kveim responsiveness as representing a specific sensitivity to some alkali-labile substance present in human sarcoidal tissue (1 3) The slowly developing intracutaneous reaction may be viewed as representing a new type of a particularly long-delayed hypersensitivity response, perhaps similar to that seen in a positive lepromin reaction It is not yet known whether K eim-responsi eness precedes the clinical onset of sarcoidosis. A fuller understanding of the nature of the Kveim reaction might well be the key to resolving the mystery which has surrounded sarcoidosis for almost 100 years.

References

1 Chase, M. W Amer Rev Resp. Dis. *84* 89—93 (pt. 2) (Nov) 1961
2 Siltzbach, L. E. Amer Rev Resp. Dis. *84* 86—88 (pt. 2) (Nov) 1961
3 Chase, M. W and Siltzbach, L. E. Excerpta Med. Int. Cong. Ser *42* 58 (Oct.) 1961

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The Nature of the Kveim Reaction

R. Kooij

I. Specificity of the Kveim Reaction

Positive Kveim reactions in diseases other than sarcoidosis are rare. The low incidence of 2 percent false positive results in controls is impressive. However we need more information about these controls. Especially about the number of granulomatous diseases and about the type of granulomatous diseases. However the number of granulomatous diseases tested and especially of those with striking sarcoid-like reaction is very limited.

A suitable disease in this respect is *tuberculoid leprosy*.

It is generally stated that the Kveim reaction in leprosy is negative. Not many results of Kveim reactions in leprosy have been published and often the number of patients tested is small and the type of leprosy is not mentioned. Especially the *type* of leprosy is important because positive reactions may chiefly be expected in tuberculoid (sarcoid) leprosy.

Most workers refer to the study of Wade (1) who tested 10 leprosy patients with two different Kveim antigens. The results were negative but one must not forget that all 10 patients suffered from the *lepromatous type*.

Only Goldman (personal communication *to Wade*), who tested in Mexico with Latapi 20 leprosy patients with a Kveim antigen supplied by Nelson, mentioned that some were of the tuberculoid type. In none of the patients the Kveim reaction was positive.

We obtained the following results with Kveim antigens in leprosy.

Material and Methods

Several Kveim antigens were injected intradermally (0.1 ml) in Bantu patients with tuberculoid and *lepromatous leprosy*.

Kveim antigen KJ obtained from Dr James, London, was prepared from an enlarged gland of a patient with sarcoidosis. This antigen had been shown clinically to be active. Kveim antigen K, *obtained from Dr Kuper* (London) was prepared from a sarcoid spleen. Clinical tests with this Kveim antigen did not reveal much activity.

From this preparation K, a preparation KC, *four times more concentrated*, was made and also a preparation KD was obtained from Kveim antigen K by extraction with chloroform and treatment with ether.

Results

Kveim antigen K, which was injected in 20 patients with tuberculoid leprosy and 15 patients with lepromatous leprosy evoked in 3 of the tuberculoid cases a papule of 2 mm diameter, in one a papule of 1 mm. Only one *lepromatous case showed a papule of 2 mm*.

The concentrated preparations from preparation K, the preparations KC and KD and the Kveim antigen KJ (from Dr James) were simultaneously injected in a number of patients with tuberculoid and lepromatous leprosy.

The readings of the reaction papules four weeks after the injection are shown in table 1

TABLE I Readings (mm) after 4 weeks with various *Kveim Antigens and Lepromin* in patients with Leprosy

No. patients	KJ	KC.	KD	Lepromin
Tuberculoid *leprosy*				
12847	5	0	5	0
12944	5	0	0	12
12933	0	5	3	5
12930	0	0	0	7
12925	2	2	0	25
12919	0	4	8	8
12918				5
12915	0	3	4	2
12897		4	4	18
12954	5	5	8	10
12845	4	5	7	
12894	2	5	5	10
12903	6	7	7	11
12904		4	6	5
12937	0	0	0	0
Average	2 4	3.0	4 0	8.0
Lepromatous leprosy				
12859	0	0	2	0
12881	0	0	4	0
12891	0	4	7	0
12908	0	0	0	1
12913	0	0	0	2
12924	0	2	1	2
12942		0	0	1
12940		0	0	0
12807		4	6	0
12813	0	0	0	0
12832		0	2	0
12910	0	0	2	0
Average	0	0.8	2 0	0.5

KJ = Kveim antigen from James.
KC = concentrated (4 times) Kveim antigen K from Kuper
KD = concentrated chloroform-ether preparation from Kveim antigen K.

For comparison the readings obtained with a lepromin preparation are given.

Four weeks after the injection, histological examination was carried out of 3 reaction papules evoked by preparation KJ a tuberculoid structure was found. Histological examination of one reaction papule from KC and one from KJ in tuberculoid cases, excised 11 weeks after the injection, showed histologically fibrosis with giant cells.

Summing up, we obtained a positive Kveim reaction with the various Kveim antigens in about a third of the patients with tuberculoid leprosy using Danbolt's criterion for positivity and in more than half of them using Nelson's original criterion[1]

Only occasionally positive reactions occurred in the lepromatous cases with the concentrated preparations. Concentration of Kveim antigen resulted in stronger reactions.

Comment

One cannot expect in all cases of tuberculoid leprosy a positive Kveim reaction. Just as in sarcoidosis the highest percentage of positive Kveim reactions is found in the active cases. In cases of inactive sarcoidosis also only one third showed positive Kveim reactions. Therefore we consider the positive Kveim reactions we obtained in tuberculoid leprosy as true positive reactions. These findings support the view that tuberculoid leprosy belongs to the syndrome sarcoidosis.

One must not forget that the resemblance between tuberculoid leprosy and sarcoidosis is very great and several times a case of leprosy has been diagnosed as sarcoidosis or vice versa. This subject will be dealt with in *a special article* which will appear in May 1964 in the British Journal of Dermatology. If you consider our positive Kveim reactions in tuberculoid leprosy as false-positives, it proves that the incidence of false-positives is very high.

At the Conference Kenzo Nobechi reported also considerable number of positive Kveim reactions in leprosy!

There is some confusion in the sarcoidosis literature about the use of the term specificity for the Kveim test. One must distinguish between *diagnostic* specificity which means that the test must be positive in sarcoidosis and negat in other diseases and *etiologic* specificity in the sense that the Kveim antigen contains *the* unknown specific agent or that the positiv test points to specific unknown agent.

I our opinion the Kveim test has *diagnostic* specificity if one considers sarcoidosis as being syndrome including tuberculoid leprosy and other granulomatous diseases with sarcoid-reaction. In the evaluation of the *Kveim reaction* quantitative factor must be taken into account. Patients with the syndrome sarcoidosis react on an average stronger *to Kveim antigen than controls*. The *Kveim test has no etiologic alue. The great*est drawback is the lack of standard prep*aration. Lepromin* also has no official standard preparation but t evokes usually reactions 2 to 3 times stronger than those of *suitable Kveim an igen.*

II. The Active Principle of the Kveim Reaction

Kveim antigen is prepared from human sarcoid tissue by extraction with saline. Several ttempts have been made to evoke sarcoid-like reactions with suspensions of normal tissues, usually with negative results.

Only Nelson (2) found that all of 11 patients with sarcoidosis, who showed positiv cutaneous reaction to the Kveim antigen, also showed typical sarcoid-like response to the injection of suspensions of normal spleen prepared in the same way as the *Kveim antigen.*

Beaumont (3) repeated the experiments of Nelson with suspension of *normal* spleen. I 10 healthy individuals no reaction was observed. Of 17 tests performed in patients with activ pulmonary tuberculosis one positiv reaction occurred.

Only one patient with active sarcoidosis was tested with positive result and from 23 patients with inactive sarcoidosis only tw showed a positive reaction. They also obtained positive reactions with suspension of normal spleen in which sand was used.

Finally Putkonen (4) obtained in patients with sarcoidosis *weak reactions, similar to* those described by Kveim, after the intracutaneous injection of saline suspensions of leukaemic human *lymph-nodes, and tuber*culous lymph glands.

Kooij et al. (5) showed that with suspensions of normal tissue positiv Kveim-like reactions could be obtained in patients with tuberculoid leprosy and healthy indi iduals, especially when concentrated suspensions were used. No reactions were evoked in patients with lepromatous leprosy. These suspensions of normal tissue therefore react in the same w y in leprosy as lepromin and *Kveim antigen prepared from sarcoid tissue.* W call this type of reaction the *lepromin pattern of reaction.* These findings were confirmed by Davey and Drewett (6) and by Leiker (7)

There is great conformity between the *preparation of Kveim antigen* and normal tissue suspensions as well as in the reaction to these compounds.

Histopathologically the reaction papules often show tuberculoid (sarcoid) structure. The active principle of these suspensions is bound *to orpuscular elements and boiling* does not destroy the activity

However not every normal tissue suspen*sion can produce sarcoid reaction. From* the twelv normal spleens tested by Nelson (8) only the preparations of 2 spleens could produce *sarcoid reaction. But one must not* forget that even preparations from sarcoid tissue often fail to produce sarcoid-reaction. Siltzbach (9) had *to reject as unsuitable al*most half of the sarcoid tissues used for making Kveim suspensions and Putkonen (4) found even 2/3 of them unsuitable.

From these results w assume that the reactions to Kveim antigen and to "normal" or at least non-sarcoid tissue suspension are basically the same.

The reason why only few suspensions of normal tissue and also why not all sarcoid tissue yield suitable preparation deserves

further investigation. Apart from qualitative differences, a quantitative factor must be taken into account. Concentration of the preparations usually results in stronger reactions.

As it is possible to obtain sometimes positive "Kveim" reactions with normal tissue suspensions and also with suspension of leukemic lymph glands it is not very likely that *only* Kveim antigen contains the active principle. It is more likely that tissue breakdown products, which are present in both Kveim antigen and in other tissue suspensions are chiefly responsible for the sarcoid reaction, just as tissue breakdown products of malignant tumours can cause sarcoid reactions in regional lymph glands.

Not much is known about the chemical complexes of these tissue breakdown products which can be produced by many agents. The concentration, the size of the particles and the chemical composition are essential elements for evoking this particular reaction pattern.

Grotepass, de Kock and Kooij (10) showed that a lipidfree preparation of normal liver could produce the lepromin pattern of reaction. Destruction of the particles by the enzyme trypsin, led to a preparation which was inactive.

These findings have a bearing on the etiology of sarcoidosis. We consider sarcoidosis an individual constitutional reaction pattern evoked by one of many agents (terrain sarcoidique). With Kveim antigen and some tissue suspensions we can test this mode of reaction.

Summary

1. The Kveim test was positive in a number of cases of tuberculoid leprosy and they are true positive reactions.
2. The Kveim reaction is a diagnostic test but has no etiologic value.
3. The active principle(s) of Kveim antigen are chiefly tissue breakdown products.
4. Sarcoidosis is a syndrome (or reaction pattern) in certain individuals to reaction products which may be caused by one or more of many agents (terrain sarcoïdique).

References

1. Wade, H. W. J. Invest. Derm. *17* 337 1951
2. Nelson C. T. J. Invest. Derm. *10*. 15, 1948.
3. Nelson C. T. Arch. Derm. Syph. Chicago *60*: 377 1949.
4. Beaumont P. M. Med. Diss. Groningen Holland. 1957
5. Putkonen T. Acta derm. vener. 25, 393, 1945
6. Kooij et al. *Dermatologica 116* 1 1958.
7. Davey T. F. and Dan[illegible] S. E. Leprosy Review 29 19 1958.
7. [illegible], D. L. Int. J. Leprosy 29 157 1961
8. Nelson C. T. J. Chron. Dis. 6. 138, 1957
10. Siltzbach, L. E. Amer. Rev. Resp. Dis. *84* part. 2 p. 100, 1961
11. Grotepass, F. W. K., de Kock, D. H. and Kooij R. *Leprosy Review 34* 129 1962

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Source of Potent Kveim Antigen[1]

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The diagnostic importance of the Kveim test in sarcoidosis has been questioned in the past, but these doubts have gradually vanished. Thus, the last international Conference on Sarcoidosis in 1960 (1) arrived at the agreement that "the Kveim test is reliable and accurate when carefully prepared from potent material". Indeed, advances have been made in the purification of antigen (2) but sufficient attention has not been paid to the sources of potent material. Williams and Nickerson (3) prepared the first test suspension from the skin lesions of patients with sarcoidosis. Later antigen has also been made from sarcoidal lymph nodes, spleen, tonsils, muscle, and even from the cutaneous papules elicited by the Kveim test (4—8). Of these, enlarged lymph nodes and spleen are the only sources of practical importance. It is difficult, however, to obtain sarcoidal spleens.

The potency of antigens prepared from lymph nodes varies greatly. It is therefore important to know what type of patient is a source of potent antigen. My study of the Kveim reaction twenty years ago (5) supplied a hint in this regard. The summary at the time included the following statement: "The most active antigens are often obtained from patients whose own Kveim test is weak." This observation, to my knowledge, has not aroused notice later. It thus seems justified to present once more the main findings leading up to this conclusion. Additional studies of this problem will also be reported.

[1] Supported by the Finnish Medical Research Council.

Previous results

My Danish material from 1943 included lymph node antigens obtained from eight patients with sarcoidosis. The standard test dose was 0.1 ml of antigen diluted 1:10 by wet weight of tissue. Tests were then followed until the reaction papule reached a diameter of 5 mm. This interval in days was taken as an inverse measure of the strength of the Kveim reaction.

Only two of the antigens were potent to the extent that they caused a 5 mm response in most sarcoidosis patients within one week. Five antigens, however, were inert and even during five months no reaction papule of any kind was produced in the same test subjects.

Tests performed with potent antigen on the patients from whose lymph nodes the antigens had been prepared showed that the two patients who were sources of potent antigen were themselves slow reactors. In one of them it took 49 days and in the other 61 days for the papule to reach 5 mm diameter. This degree of response was obtained within a week in the other antigen patients.

Continued studies

The investigation was later continued in Finland with suspensions prepared from peripheral lymph nodes of eight new sarcoidosis patients. Histologically all these lymph nodes showed a sarcoid structure. In addition, five patients had bilateral hilar lymphomas, three showed signs of sarcoidosis in the lungs, four in the skin, two in the bones, two on the mucous membranes, and one had iritis. The prep-

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TABLE I Responses of nine test patients with sarcoidosis to Kveim antigens prepared from sarcoidal lymph nodes of eight patients

Antigen No.	Test patients with sarcoidosis 1	2	3	4	5	6	7	8[3]	9
51	+ 180[1]	+ 103							
52		+ 11							
53	+ 75	+ 23	+ 74	+ 8	+ 51	+ 121	+ 75	81	+ 71
54		+ 15	+ 20	+ 4	+ 14	+ 13	+ 16	7	
107			+ 44	+ 39	− 131	− 107			
108			+ 103	+ 10	− 131	+ 62	+ 91		
110				+	+ 4	+ 5	+ 18	14	+ 12
111							+ 19	16	+ 21

[1] A 5 mm reaction papule in 180 days.

No reaction papule during a follow-up period of 131 days.

[3] A slow reactor. Results based on 4 mm papules.

aration of antigen, the testing technique, and the reading of results were consistent with those in the earlier study.

Table I shows the responses of nine test patients with sarcoidosis to these suspensions. The potency of the suspensions could not be classified into two separate groups as was the case with the eight antigens in the previous study. They rather consisted of a series showing variable potencies.

For the purpose of comparison, ratios were computed indicating the potency of each antigen. The potency of antigen 53 (= Y_{53}), tested on all nine patients, was given an arbitrary numerical value 10. The reactions obtained with this antigen were then compared, using reaction time (= X) as measure, with those obtained with antigen 54 in the seven patients tested with both these antigens. The mean of the seven numerical values, $\bar{Y}$ = 48, as calculated from the equation

$$\frac{X_{53}}{X_{54}} \times 10 = Y_{54}$$ was then adopted as the definitive value for the potency of antigen 54. In contradistinction to the reaction time, this value is the greater the more potent the antigen. The other antigens were then compared with the above two so that calculation of their potency was based on a greater number of observations. In the case of patient No 8 the values, exceptionally, were calculated on the basis of a 4 mm papule. In this slowly reacting patient the follow up period was too short for all reaction papules to reach the 5 mm limit.

In table II the suspensions are arranged according to their calculated potency values. This table also shows the antigen patients own response to antigen 53 and to the Mantoux test with old tuberculin. Antigen 53 was selected because six of these patients had been tested with it so it was necessary in only two cases to calculate the response to this antigen from the results obtained with other antigens.

Statistical analysis of the results shows that there is a reverse mutual dependence between the potency of the antigen prepared from lymph nodes of a patient with sarcoidosis and his own response to the Kveim reaction: a patient who responds strongly is a source of weak antigen, and vice versa.

Another observation worth noticing emerges from table II. The patient who was the

Kendall rank correlation coefficient = −0.91, $P < 0.001$, when excluding suspensions 51 and 52 = −0.97, $P < 0.01$. This statistical analysis was carried out by Heimo Jarvinen, M. Sc.

Table II. Potency values of antigens prepared from lymph nodes of eight sarcoidosis patients compared with the patients own response to Kveim test and old tuberculin

Antigen No.	Potency of antigen 53	Response of patients to Antigen	Mantoux (T U of O.T)
51	4	+ 13	— 100
107	6	+ 8	— 100
108	9	+ 51	— 100
53	10	+ 75	
111	30	+ 73	— 100
52	39	+ 138	— 100
54	48	209	+ 10
110	93	— 149	+ 1

Obtained by calculation (see text).

In 209 days 4 mm papule only

source of the most potent antigen (No. 110) responded to as little as 1 T U of old tuberculin and the patient next in order in this respect (No. 54) to 10 T U By contrast, those patients whose tissue suspensions elicited weak Kveim reactions were tuberculin-negative even with 100 T U

Discussion

The source of potent Kveim antigen seems to have aroused but little interest no studies of this particular question have come to my attention. It is usually stated that the tissue used for preparation of the suspension must show histologically sarcoid structure. This claim should not be carried too far by excluding tissues with "fibrous étendue" as has been done (9); one of my most potent antigens (antigen 1 previous study) was derived from lymph node which was almost totally converted into fibrous tissue (5) Only small scattered groups of epithelioid cells could still be found. The present study did not reveal any correlation between the duration of patient disease and the potency of the antigen obtained from his lymph nodes.

The interesting fact that the most potent antigens are obtained from patients with weak Kveim reactions arouses more questions than can be answered, because of our incomplete knowledge of the nature of the Kveim reaction. In practice however this observation is valuable because it facilitates the obtaining of potent antigen. The number of false positive test results is reduced by using the most potent antigens, which can be sufficiently diluted. These antigens are also of value in checking the negative test responses evoked with weak antigens.

It is of interest that the two most potent antigens were derived from sarcoidosis patients who were more sensitive than the others to tuberculin. This is a new clue which may be of value in attempting to clarify the nature of the Kveim reaction and the relationship of sarcoidosis to delayed hypersensitivity and especially to tuberculosis.

Summary

The present communication is based on investigations of Kveim antigens prepared from lymph nodes of sixteen sarcoidosis patients and of the reactivity of these patients to the Kveim test. The results show that the most potent antigens are obtained from patients who themselves respond weakly to the Kveim test.

References

1 Smith, D. F Amer Rev resp. Dis. 84/5 174 1961
2. Chase, M. W Amer Rev resp. Dis. 84/5: 86—88 1961
3. Williams, R. H. and Nickerson, D. A. Proc. Soc. Exper Biol. & Med. 33 403—404, 1935.
4 Kveim, A. Nord. Med. 9 169—172 1941
5. Putkonen, T Acta derm.-venereol. 23 suppl. 10, 1943.
6. Danbolt N. Acta derm.-venereol. 28: 151—157 1948.
7 Nelson, C. T Arch Derm. Syph. 60: 377—438, 1949
8. Siltzbach, L. E. and Ehrlich, J C. Amer J Med. 16. 790—803, 1954.
9 Turiaf, J. Presse Méd. 70: 2017—2020, 1962.

From the Pathology Dept., Public Hospital, Wellington, New Zealand

Remarks on "The Nature and Significance of the Kveim Test"

J D Reid

Our interpretation of the Kveim test has been considerably influenced by experience with skin reactions to injections of heat-killed intact organisms. The response seen here must approximate to the sum total of body reactions to an infection much more closely than response to purified derivatives or organisms. In experiments conducted over the past two years, chiefly using mycobacterial suspensions, both in hospital patients and in sensitized animals, it has become clear that the skin reaction in such a test system is biphasic in character After the expected delayed reaction read at 48—72 hours, the wheal decreases as usual but then becomes stationary in diameter and develops in height to form a lump which persists for many weeks and which may ulcerate. This double reaction has been seen with all mycobacterial types tested. It has also been produced by organisms such as Nocardia or even Gram negative bacilli of a strain of Brucella ovis. We have called the second phase the "late" reaction as opposed to immediate and delayed response In its production, appearance, and timing, the late reaction corresponds to Koch accelerated tubercle reaction as obtained with very small numbers of live or dead organisms and it is also very much like the Mitsuda reaction in leprosy Macroscopically and again in time of appearance it is very much like a positive Kveim reaction, which is a similar indolent lump and which may occasionally be seen to ulcerate. It is not merely a foreign body response since it is not obtainable in this form except in appropriately sensitized animals, although a foreign body component may enter into it. Histologically this late reaction differs from the delayed phase, being much more granulomatous in character Although the lesions we have produced usually show a diffuse non-specific type of infiltrate with numbers of polymorphs and evidence of a somewhat acute inflammatory reaction, the response to a low dose of organisms can result in occasional follicles and giant cells, and could sometimes, if circumstances were appropriate, be interpreted as a positive Kveim test. The late phase can be obtained by itself and without a delayed reaction either by desensitizing the subject with massive doses of old tuberculin before testing or by giving a test injection of organisms insufficient to produce the delayed reaction. Under these circumstances the characteristic lump will still appear It has been our hypothesis (and one must have a hypothesis to work on) that sarcoidosis is probably an infective process due to an unidentified organism and in this context we consider that the Kveim test may represent the late phase of a reaction either to whole organisms or to their antigenic derivatives modified by phagocytosis or bound to connective tissue components. The Kveim test has obvious similarities to the experimental situation in which low dosages of organisms give a late reaction only It also resembles the situation in leprosy where crude tissue extracts containing lepra bacilli give late reactions at 4—6 weeks while refined material

Skin reactions.	Delayed 48 hour	Late 4 week
Tuberculosis	Mantoux	Koch accelerated tubercle
Leprosy	Fernandez	Masuda
Sarcoid	Williams-Nickerson	Kveim

given many more 48 hour (Fernandez) responses and fewer at the later time. Since Williams and Nickerson originally read their test at 36 hours, delayed reaction does seem possible in sarcoidosis although I am disturbed by the fact that no one else, and Dr Siltzbach in particular has produced it. In our experience the positive K eim test has been obtained only from test material made from sarcoid tissue and only in patients with the clinical syndrome of sarcoidosis. Because of these two facts w feel that the test is specific but if viewed as an example of type of skin reaction — the late phase — probably specific only in the sense that Mantoux test is specific, i. e., cross reactions might occur with related organisms.

If the premise is true that the positiv Kveim test is late phase of reaction to some organism, there are important corollaries. Failure *to obtain positive Kveim tests in* cases of tuberculosis, and conversely failure to produce late phase reaction to acid-fast organisms in cases of sarcoidosis unless Mantoux positive, indicate that the causal agents are not at all closely related antigenically Further *the organism if any causing sar*coidosis, need not be mycobacterial or acid-fast in nature, but could be any one of those which can produce biphasic or late skin reaction.

From the Pathology Dept., Public Hospital, Wellington, New Zealand

Remarks on "The Nature and Significance of the Kveim Test"

J D REID

Our interpretation of the Kveim test has been considerably influenced by experience with skin reactions to injections of heat killed intact organisms. The response seen here must approximate to the sum total of body reactions to an infection much more closely than response to purified derivatives or organisms. In experiments conducted over the past two years, chiefly using mycobacterial suspensions, both in hospital patients and in sensitized animals, it has become clear that the skin reaction in such a test system is biphasic in character. After the expected delayed reaction read at 48—72 hours, the wheal decreases as usual but then becomes stationary in diameter and develops in height to form a lump which persists for many weeks and which may ulcerate. This double reaction has been seen with all mycobacterial types tested. It has also been produced by organisms such as Nocardia or even Gram negative bacilli of a strain of Brucella ovis. We have called the second phase the "late" reaction as opposed to immediate and delayed response. In its production, appearance, and timing the late reaction corresponds to Koch's accelerated tubercle reaction as obtained with very small numbers of live or dead organisms and it is also very much like the Mitsuda reaction in leprosy. Macroscopically and again in time of appearance it is very much like a positive Kveim reaction, which is a similar indolent lump and which may occasionally be seen to ulcerate. It is not merely a foreign body response since it is not obtainable in this form except in appropriately sensitized animals, although a foreign body component may enter into it. Histologically this late reaction differs from the delayed phase, being much more granulomatous in character. Although the lesions we have produced usually show a diffuse non-specific type of infiltrate with numbers of polymorphs and evidence of a somewhat acute inflammatory reaction, the response to a low dose of organisms can result in occasional follicles and giant cells, and could sometimes, if circumstances were appropriate, be interpreted as a positive Kveim test. The late phase can be obtained by itself and without a delayed reaction either by desensitizing the subject with massive doses of old tuberculin before testing or by giving a test injection of organisms insufficient to produce the delayed reaction. Under these circumstances the characteristic lump will still appear. It has been our hypothesis (and one must have a hypothesis to work on) that sarcoidosis is probably an infective process due to an unidentified organism and in this context we consider that the Kveim test may represent the late phase of a reaction either to whole organisms or to their antigenic derivatives modified by phagocytosis or bound to connective tissue components. The Kveim test has obvious similarities to the experimental situation in which low dosages of organisms give a late reaction only. It also resembles the situation in leprosy where crude tissue extracts containing lepra bacilli give late reactions at 4—6 weeks while refined material

Table I. The correlation between pathologist A's first and second reading

		Second reading			
		+	?	—	Total
First reading	+	297	49	0	346
	?	10	110	33	153
	—	2	18	882	902
	Total	309	177	915	1 401

Table II. The correlation between pathologist A's and pathologist B' reading

		Pathologist B			
		+	?	—	Total
Pathologist A	+	212	41	1	254
	?	23	49	12	84
	—	4	39	182	225
	Total	239	128	195	563

represent sections which were interpreted the same way both times — either positive, questionable, or negative. It can be seen that 92 per cent of the sections were read consistently and the remaining 8 per cent were read inconsistently however direct reversals between positive and negative result were rare.

Reader A has thus been proved able to read rather consistently. But, this is not sufficient for clinical work with the test. The readings of *one pathologist must be consistent with those* of other pathologists. In order to test whether this was the case, a comparison was made between pathologist A and another pathologist, here called B. A sample comprising 563 sections was sent to B, who read them without any knowledge of A findings. Table II compares the readings of the two pathologists. The bold-faced figures show the cases in which they agreed, a total of 79 per cent of the sections. Thus, the two readers disagreed on the interpretation of 21 per cent of the sections, even if direct reversals between positive and negative result were rare. The frequent *disagreements in the histologic interpretation*

Table III. Classification of biopsies according to findings in central five sections

	Findings in the 5 sections		Number of patients
Simple responses	positive	+ + + + +	11
	negative	— — — — —	15
Mixed responses	example	+ — + ?	28
Total			54

must have practical consequences, e. g. introduction of special training for pathologists who should read the sections.

The second assumption mentioned in the introduction is that the histo-pathologic findings are identical throughout the entire biopsy. In order to test this assumption, five sections were taken from the middle half millimeter of each biopsy to see whether they gave unanimous results.

Table III shows that simple responses, with either all five sections positive or all five sections negative, were observed in half of the patients. A mixed response was observed in the remaining patients. The one patient whose biopsy has been used as an *example of this group in Table III could have* been classified either as positive, negative, or questionable if only one section had been ex*amined. Admittedly not all patients in the* mixed group had so pronounced a variation, but in all of them the diagnosis would depend on the number of sections examined. This means that if only one or two sections were read, the diagnosis would become a matter of chance.

The result of the present study indicates that a further standardization of the Kveim test is desirable.

References

1. Editorial, The Lancet, 1962 I p. 1107
2. Siltzbach, L. E. and Ehrlich, J. C. Am. J. Med. 16: 790, 1954.
3. Siltzbach, L. E. Am. Rev. Resp. Dis. 84 No. 5, Part 2, 89, 1961.

From the Danish Tuberculous Index, Copenhagen, and the Pathology Institute, Holbæk, Denmark

On the Standardization of the Histo-Pathologic Reading of the Kveim Test

J Ringsted and J B Ferebee

The introduction of histologic examination in the Kveim test has apparently made the test more reliable. It is now generally accepted that a Kveim test should only be considered positive when epithelioid cell granulomas have been demonstrated in a biopsy from the test site. Under these conditions the test is now accepted as safe, simple and specific (1)

However since the histologic reading is based on an estimate of a morphologic quality it will only prove reliable if the following two assumptions hold true (1) that pathologists are highly consistent in their diagnosis of an epithelioid cell granuloma (2) that the histologic picture is the same throughout a Kveim biopsy

The Danish Tuberculous Index has undertaken a study to investigate the validity of these two assumptions.

The Kveim test was performed on patients who were suspected of having sarcoidosis. Antigen from the Rockefeller Institute Lots 7 and 8, was kindly placed at our disposal by Dr Louis E. Siltzbach, New York. Each patient was tested with 0 2 ml of antigen, given intradermally on the front of the thigh, 20 cm above the upper border of the patella. The test was examined after 5—6 weeks, and a biopsy was made if an induration was present.

The present study is based on biopsies from 54 patients. In each case the whole biopsy was cut into sections 5 micron thick, and every 20th section was submitted to histological examination. This gave a total of 1401 sections and an average of 26 sections per biopsy All sections were stained with hematoxyline and eosine. The microscopic examination was made with both ordinary light and polarized light.

The histologic findings were classified according to criteria established by Siltzbach & Ehrlich (2) and Siltzbach (3)

(a) *Positive result* Sections containing at least one granuloma composed of epithelioid cells, with or without giant cells. A simultaneous occurrence of densely packed epithelioid cell infiltration in another area of the section, of lymphocytes, of other inflammatory cells, or of fibrinoid necrosis did not influence the interpretation.

(b) *Questionable result* Sections in which it was doubtful whether or not epithelioid cell granulomas as defined above were present. This group also included sections with granulomas which could not be classified with certainty either as specific granulomas or as foreign body granulomas.

(c) *Negative result* Sections without granulomas as defined above This group includes sections with unspecific inflammation or with foreign body granulomas.

The first problem was to see how consistently a pathologist could read the sections. The 1401 sections were read twice in random order by one of us, here called A. *Table I* shows the correlation between the two readings. The bold-faced figures along the diagonal

The study was supported by grant No. AI-01817 from the National Institutes of Health, U.S.A.

colleague test suspension which purported to be one of "normal" spleen. That "normal" suspension produced granulomas in everyone, sarcoid and nonsarcoid subject alike. It became clear to me that unless the so-called "normal" tissue suspensions are tested in well persons and subjects with diseases other than sarcoidosis, one cannot really say whether they contain some non-specific granuloma-inciting material.

No one can question any longer the test suspensions made of tuberculous lymph nodes, even leukemic lymph nodes, and so-called "normal" lymph nodes may on occasion, elicit in some individual intracutaneous granulomas which look like positive Kveim tests. *But to my knowledge,* none of these various tissue suspensions possesses the specificity that properly screened and validated sarcoidal tissue Kveim test exhibits. The trouble is that granulomas which these nonsarcoidal tissue suspensions evoke are not limited to patients with sarcoidosis but can be observed in patients without sarcoidosis and hence are not to be considered as Kveim test suspensions.

I was also interested in Prof. Putkonen beautiful slides. I am personally grateful to Prof. Putkonen since my work has followed along the path of his pioneering studies. At one point, in his talk, I observed that Prof. Putkonen returned to his original scheme for determining whether Kveim test was positive or negative and for gauging how strongly positive it was. He employed the interval of days after injection it took for the Kveim papule to reach diameter of 4 mm. By this method, sometimes almost 200 days may pass before the test can be called positive. In 1954, (Am. J. of Med. Vol. 16, pp. 790—803) I suggested that *obligatory* biopsy of the Kveim papule be made at *four to six weeks, preferably at six* weeks. In this way the specific histo-pathologic pattern could be confirmed and the time for reading the test, too long even *now* could be shortened. Six weeks obligatory biopsy has been the procedure that Prof. Putkonen and I have been using in our collaboration in the International Kveim Study. As for Dr. Rimpela's painstaking study of the Kveim test, I shall comment on it briefly during the session on the International Kveim Study tomorrow.

Dr. Kooij. There is some confusion in the sarcoidosis literature about the use of the term specificity for the Kveim test. One must distinguish between *diagnostic* specificity which means that the test must be positive *in sarcoidosis* and negative in other diseases, and *etiologic* specificity in the sense that the Kveim antigen contains *the* responsible (specific) agent and that the Kveim test points to specific etiology. In my opinion the *Kveim test has only diagnostic significance* for the *diagnosis* sarcoidosis but has no etiologic significance. It does not give information about the etiology of sarcoidosis.

Dr. James. Those people who have no Kveim antigen continue to disparage the Kveim test. But you Dr. Kooij are in the happy position of being able to get the test to work without using sarcoid tissue. Many people throughout the world must be curious and if it is indeed possible to do so, then the scarcity of sarcoid tissue antigen becomes unimportant.

Dr. Kooij. The tissue was obtained from healthy people (died by accident) and the tissue looked normal and it was certainly *not* sarcoid tissue.

Our results show that with others than sarcoid tissue sarcoid reaction can be evoked.

Not every normal tissue suspension can produce sarcoid reaction. From the 12 normal spleens tested, Nelson found that only the preparations from 2 spleens could produce sarcoid reaction. Nelson calls this rare occurrence. But one must not forget that even preparations from *sarcoid* tissue often fail to produce sarcoid reaction. Siltzbach had to *reject as unsuitable almost half of the* sarcoid tissues used for making Kveim suspensions and Putkonen even found 2/3 of them as unsuitable.

Dr James I am very sorry that Dr Kveim is not with us today He is charming gentleman and so modest about the important work he has done. He would have been delighted to hear the continuing interest in his pioneer work.

Dr Lofgren Dr Kooij and Dr Reid touched upon an essential question in this connection th nature of the Kveim reaction. We already knew that sarcoid lesions can be provoked by injecting sarcoid tissue material, but other materials may also be used. Present here to-day are, for example, Dr Lemming and Dr Warfvinge who many years ago provoked similar cutaneous lesions by injecting BCG-vaccine and killed tubercle bacteria respectively According to Dr Kooij t is likewise possible to use normal tissue for this purpose. However the most important question for me is why do sarcoidosis patients react in this peculiar way? And are there other diseases which behave in the same manner viz. that you can take inactivated specific tissue — or non-specific tissue — and by intracutaneous injection reproduce specific lesions in patients suffering from the disease in question? If you could tell me three or four or fi e — or still better ten — such diseases, then I think I would be able to tell you what sarcoidosis eally is. As far as I ha e understood it, lepra may be a disease of this kind, but I would like to know more diseases of this type.

Dr Rakower K elm test as well as lepromin test were performed by us on 20 patients with tuberculous or lepromatous leprosy None of them was K elm positive and none presented sarcoid reaction after the lepromin test None of the 42 patients with miscellaneous pulmonary diseases (tuberculosis, bronchiectasis, lung cancer) was Kveim positive.

On the ther hand 23 of the 25 patients w th sarcoidal bilateral hilar lymphoma syndrome were K eim positive (92) while they were negative to Fred nd Leishmania tests. The loss of activity to Kveim ntigen after period of one to tw years in the same patients par lleled with roentgenological disappearance of the hilar lymphoma. In the later stages of sarcoidosis the Kveim test yielded only 60 of positive results.

I used 5 different antigens tw made by my self, Dr Siltzbach provided us the three others. There was no significant difference n the intensity of the reaction between the Israeli and th American antigens.

In our experience the Kveim test is specific for sarcoidosis and one of the most reliable biological tests in clinical practice.

Dr Reid I can partially answer Dr Löfgren question and suggest an experimental model. If you take tuberculous lymph nodes from infected animals, inactivate by heat and inject suitably small doses into sensitised animals, then you may obtain reactions which are microscopically comparable to the Kveim test.

Dr Nobechi The members of the Sarcoidosis Research Committee in Japan applied the Kveim antigen, generously supplied by Dr Siltzbach, to 48 sarcoidosis or suspected cases. And our findings as to histological examinations of the reactions were compared with those by Dr Siltzbach. The results of the double readings, thus, performed on other sides across the Pacific Ocean concurred in 40 cases out of 48 instances, the rate of agreements being 83.5 % which was almost coincident with the agreement rate of double readings, as just reported by Dr Ringsted. These rates of agreements of double readings should not be regarded so poor when compared with the unexpectedly great discordance in the results of double readings of the chest X-ray films experimented by the leading American phthisiologists. However I would concur with Dr Ringsted to desire to have the Kveim test standardized, so as its reliability would still be improved.

Dr James Good go on, I would much rather hear from some of the people who criticise the K eim test. There are too many people who hav already spoken of its alue. There are two spheres n the study of sarcoidosis where emotion over takes us one is when people discuss ts relation w th tuberculosis and the other is on the value of the Kveim test. For the rest of this discussion I would prefer people to discuss the snags surrounding the K eim test because it might help us resolve some of them in the future.

Dr Siltzbach Prof Kooij injected normal" tissue suspensions in place of K eim suspensions into the skin of three patients w th sarcoidosis and five patients suspected of ha ing sarcoidosis H observed gross papules in all eigh patients after four weeks and all of the papules showed microscopically a more or less tuberculoid pattern on biopsy I have not encountered normal tissue suspension which could be substituted for validated K eim suspension as diagnostic agent in sarcoidosis. I ha tried a half-dozen normal pleen and normal" lymph node suspensions and have tested them in wide ariety of pa tients w th and without sarcoidosis. Almost all of these normal tissue suspensions proved t be quite inert or nonspecific. I did recei from a

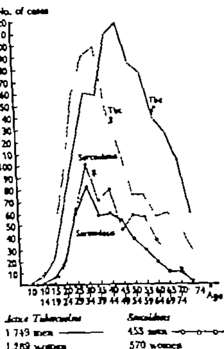


Fig. 1. The relationships between pulmonary sarcoidosis and active pulmonary tuberculosis detected by RP at the first Swedish mass chest surveys, 1950—60.

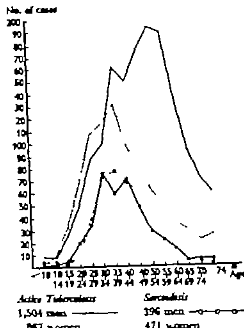


Fig. 2. The same relationships as in Fig. 1 at the repeated second mass chest surveys, 1953—61.

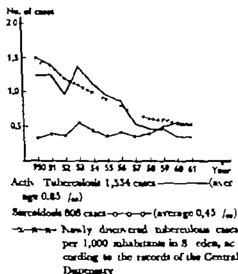


Fig. 3. The relationships between pulmonary sarcoidosis and active pulmonary tuberculosis detected by RP at the mass chest surveys of the Swedish armed forces, 1950—61

of sarcoidosis in considerably younger categories than lung tuberculosis, and that the frequency of sarcoidosis seems to be practically the same amongst both men and women. Of special interest is the tendency shown in Fig. 3 which in curve-form represents the incidence of pulmonary sarcoidosis in military material, mainly new recruits about 20 years of age, in relation to ditto incidence of pulmonary tuberculosis. The sarcoidosis curve rises steadily and at the year 1961 intersects the strikingly falling tuberculosis curve. This should signify that while tuberculosis is in marked, constant decline as consequence of our successful measures, sarcoidosis is becoming disease of progressive development with closely related dominance in younger people.

EPIDEMIOLOGY OF SARCOIDOSIS

Moderator: SVEN LÖFGREN

Methodological Aspects of Mass Chest Radiography

From the Swedish State Mass Chest (RP*) Center Stockholm

Case Findings and Roentgen Diagnostics of Pulmonary Sarcoidosis

CARL WEGELIUS

Pathological pulmonary changes in the form of hilar gland enlargement and parenchymal infiltrations, as we know are one of the most significant manifestations of sarcoidosis. Since these symptoms are primarily visualized and stated by aid of the roentgen picture, the roentgenographic procedure has become one of our most important diagnostic factors in sarcoidosis case findings. This situation is accentuated further by the often asymptomatic or "silent" course of sarcoidosis. Here the disease periodically appears as pathological pulmonary lesions without th patient suffering from distress which would lead him to seek medical care

Because sarcoidosis can thus exist hidden in an individual who feels well, its incidence, according to our information, should be considerably greater in countries where the entire population, regardless of any pathological symptoms, is the object of medical control, i.e., chest roentgen (RP) in mass surveys. Primarily it would for this reason be expected that the sarcoidosis incidence increases sharply when mass RP surveys are introduced in a country where they were not earlier carried out. This presupposes further the purposeful attention which must be devoted to the diagnosticating of sarcoidosis, knowledge of the roentgen diagnostic criteria of sarcoidosis, and finally an optimal technique for the roentgenographic recording itself. Since these goals for the present continue to be met quite differently in the mass chest RP carried out in various countries, the results naturally are correspondingly ununiform and even misleading when one attempts to make comparative evaluation of the sarcoidosis incidence.

Against this background, certain statistical data on pulmonary sarcoidosis in Sweden, discovered at mass chest RP can be of interest. This diagnostic has for several years been *performed with special attention to all the* factors which promote the roentgen diagnostic case findings of this particular disease. Every case of reported pulmonary sarcoidosis in this material is verified by thorough, detailed examination and follow-up at chest clinics, sanatoria, etc. These are presented in Figures 1 and 2 both in absolute numbers and in proportion to other RP findings, especially pulmonary tuberculosis. This method of reporting the incidence of pulmonary sarcoidosis in its relative proportion to a closely related type of disease, which is so well controlled and investigated, appears to have both *theoretical and practical value. The figures* in the tables speak for themselves. In this connection, we should like to emphasize the typical age distribution, with the dominance

*RP (abbreviation for Radiophotography) — internationally accepted term for the photofluorographic procedure.

and on diagnostic interest for and knowledge of the diagnosticating of pulmonary sarcoidosis.

An improved surveyal of the incidence of pulmonary sarcoidosis and the resulting increased diagnostics can thus be best effected through purposeful mobilisation of the mass chest RP surveys even for the case-finding of *this disease—in addition to its recognized* utilization for the detection of pulmonary tuberculosis, cancer and cardiovascular conditions. This can be achieved without extra costs from the same RP material if we only have the good will and knowledge.

Acknowledgements:

The diagrams in Figures 1—3 were kindly put at disposal by Dr. Syh. Wigström, the Swedish State Mass Chest Center

The roentgenologic criteria of sarcoidosis.

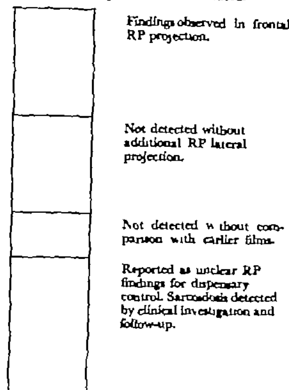


Fig. 4 The proportions between the different diagnostic criteria which enabled the diagnosis of sarcoidosis.

It is indicated above that the diagnostic gain in sarcoidosis detection made through *chest roentgenography is* dependent upon the recording technique used, which gives varying information on the incidence of sarcoidosis depending upon its greater or lesser effectiveness. This concerns, of course, mass chest RP to the same extent as conventional, large size, direct roentgenography — According *to our experiences in Sweden, the routine PA* frontal chest projection often is not sufficient for the detection of pulmonary sarcoidosis so that the case becomes subject for the necessary further examinations which are needed for a definitive diagnosis. Enlargement of the hilar glands, the most important and most common roentgen diagnostic *criterion for* pulmonary sarcoidosis, can be so much better determined in the lateral projection, where under- and overdiagnosis can be avoided, that this ought to be an obligate addendum in all mass chest surveys. Through under-diagnosis, too many positive cases are not observed and thus are lost to our consideration. Over diagnosis, on the other hand, means that dispensaries, *chest clinics*, etc., entrusted with clinical follow-up control are overburdened with analyses of so many unnecessarily remitted negative cases that the whole mass survey procedure is put to discredit.

The importance of the lateral projection in the detection of sarcoidosis in mass chest RP is illustrated in fig. 4. In our pulmonary sarcoidosis material represented here thus only about one-third of the cases could be discovered from the frontal projection alone, whereas the same amount again could be found thanks to the lateral projection. Analogously the sarcoidosis incidence reported in mass chest RP surveys in countries where lateral projection is not routinely taken will be much lower than what it actually is.— The remaining two sarcoidosis diagnoses can, as is evident from the schematic arrangement, be made first after repeated examinations with comparison of subsequent recordings from different occasions, indicating the course of the procedure, and/or with help of various clinical investigations. Within these two last groups are found the majority of parenchymal infiltrations in the lungs themselves, while the two previous groups include primarily the hilar adenitis cases with their specific differential diagnosis against tuberculosis and diverse systemic diseases.

Conclusions

The *mass chest* RP *surveys* account for a very appreciable amount of case-finding in pulmonary sarcoidosis.

The reported incidence of this disease in individual countries thus becomes dependent upon if and when RP surveys are introduced.

The incidence of sarcoidosis depends however *not only upon whether* or not RP surveys for the most part are carried out, but also upon *the way in which the roentgenographic* recording is performed—especially if lateral chest projections are taken or not—

TABLE I. Pathologic changes suggested during tw independent readings of series of photofluorograms

Hilar region

2nd reading	1st reading Neither	Right	Left	Both	Total
Neither		1	—	3	4
Right	—	1	—	—	1
Left	—	—	2	1	3
Both	—	—	—	4	4
Total	—	2	2	8	12

Lung fields

2nd reading	1st reading Neither	Right	Left	Both	Total
Neither		—	1	3	4
Right	1	1	—	1	3
Left	—	—	—	2	2
Both	—	—	—	13	13
Total	1	1	1	19	22

make diagnostic error; first of all, the appearance of the technically deficient photofluorogram may be assumed to be caused by respiratory sarcoidosis. Secondly there is the possibility that if the two conditions—underdevelopment and sarcoidosis—happen to coincide, the pathological abnormalities are either camouflaged or regarded as being the result of the inadequate photographic technique.

A tentative working hypothesis was established to account for the result of the two independent readings. According to this, reader is liable to assess technically inadequate photofluorograms in the w y described abov as the first possibility until he becomes accustomed to the technically inadequate photofluorograms. When habituation to this type of film has occurred, an unconscious, and therefore uncontrolled, perceptual compensation takes place, and subsequently depression of the diagnosis of sarcoidosis occurs.

This hypothesis might hav important practical implications, and it was decided to examine the question in further detail. The same reader made a third reading of the series of photofluorograms, and he was given the task to try to compensate consciously every time he thought that technically insufficient photofluorogram might contain sarcoidal changes. The reading was independent in so far as the reader was kept unaware of the results of the tw initial readings but he was of course influenced by the instructions given to him prior to the reading. When the results of the third reading were compared with the results of the two preceding ones, t was found that they constituted a sort of average between the first and second. However w do not know which of the three readings gives the best estimate of the actual number of patients in the series, and for this reason w cannot recommend specific instructions in the case of technically insufficient photofluorograms. However the results of the study indicate the necessity for the strictest technical discipline. If morbidity studies on respiratory sarcoidosis have to be based on photofluorograms, the reader should never accept technically inadequate pictures.

From the Danish Tuberculosis Index, Copenhagen, Denmark

Technical Inadequacy of Photofluorograms as a Source of Error in the Diagnosis of Respiratory Sarcoidosis

Helge Nielsen, Ole Horwitz and Erik Wilbek

During a pilot study on the correlation between the Kveim test and the X ray findings in a series of patients who were suspected to have respiratory sarcoidosis, it was incidentally found that an underexposure or underdevelopment of the films systematically influenced the diagnostic assessment of the photofluorograms. This technical inadequacy apparently resulted in a diagnostic reeling with regard to sarcoidosis, and the Danish Tuberculosis Index is now planning a more thorough and comprehensive investigation of the practical consequences of the problem. But, because worldwide and systematic studies on the morbidity of sarcoidosis based on photofluorograms might be imminent, it is thought that a brief note about the pertinent results of the pilot study is justified at this time, even if the material is small.

The pilot study comprised 71 patients thought to have or have had respiratory sarcoidosis and admitted to various chest clinics in Denmark. Photofluorograms, 70 × 70 mm, were available for 39 of the patients, and full sized roentgenograms for the remaining patients. The reader of the films knew that the trial concerned patients with sarcoidosis. In order to decrease this bias, the two series of films were mixed randomly with a number of films of corresponding type from either normal persons or patients with pulmonary diseases other than sarcoidosis. The exact number of those films was not known by the reader but it should be stated that there were about twice as many of these as there were study films"

The resulting series of roentgenograms and photofluorograms were read independently at two different times by one of us (H. N.). The results of the two sets of readings were given on separate cards so that neither the reader nor the assistant secretary knew the result of the first reading. It was also impossible to identify the films during the readings.

The results of the two independent readings of photofluorograms showed that far more pathological changes were suggested during the first reading than during the second reading, cf. Table I. This observation was so conspicious that the photofluorograms were assessed from a purely technical point of view. It was found that the series contained several photofluorograms which were underexposed or underdeveloped, and that the photofluorograms which caused the asymmetrical distribution all belonged to that group.

Apparently this inadequate technique influences the radiographic image in the same direction as respiratory sarcoidosis, which is generally assumed to give rise to diffuse and symmetrical changes in the lungs and in the hili. There is a lowering of the general density level in both instances, creating purely photographic similarity. This similarity in appearance gives the reader two chances to

The study presented at the Third International Conference on Sarcoidosis, Stockholm 1963, was supported by grant No. AI-04817 from the National Institutes of Health, U.S.A.

Fig 1

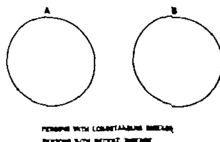


Fig. 2

what is considered an activ case, and what is cured case, the rates are meaningless. Fig. 2 is an illustration of the difficulties encountered in the interpretation of incidence figures. T the left, in the figure A, w see the situation present in population before mass examination. There are many persons who are ill, some with long-standing disease indicated by + and others with new or recent disease indicated by The rate found in this population at the time of the first examination is actually prevalence rate. T the right, in the figure B, w find the situation present in the following years where one finds only those cases which have arisen since the last examination indicated by the The problems which arise in the interpretation of incidence rates stem from the fact that great part of the population is screened annually as represented by situation B and, in this screening, there are also new groups which correspond to situation A. Thus w must have clear knowledge on the extent of the examination of population and the changes in the screening procedure before w can inter

8—641244

From the Danish Tuberculosis Index, Copenhagen, Denmark

Epidemiological Research in Sarcoidosis

Ole Horwitz

It is only in recent years that epidemiology has been recognized as a special field of medical research. Before this time, it was considered a part of the ordinary vital health statistics—a part that only involved an accumulation of numbers that would reflect the incidence of and mortality from the given disease. Today epidemiology is, in fact, a section of medical research on the same level as bacteriology or pathology with its own specialists, methodology etc. The Danish Tuberculosis Index is an example of the epidemiological research institution. The Index, in cooperation with the Tuberculosis Research Section of the USPHS, has been actively working on a model which may be used to show the dynamics of the epidemiology of tuberculosis. The details of this work will be found in Horwitz, O & Palmer C. E. Epidemiological basis of tuberculosis eradication. II. Dynamics of tuberculosis morbidity and mortality Bull. World Health Organ. 1964 30 609 Although this model, shown as Fig 1 was originally intended as an illustration of the flows and pools for tuberculosis, it was also considered applicable to the epidemiology and natural history of other chronic diseases.

The dynamics in tuberculosis morbidity and mortality may be visualized as involving four main pools of persons connected with continuously flowing streams. The largest pool is the general population, composed of persons who have never had tuberculosis the three smaller pools are composed of the active cases, the previous cases, and the deceased cases, whether active or previously active at the time of death. Two streams flow into the Pool of Active Cases, the first-time cases coming from the general population, and the relapsing cases coming from the Pool of Previous Cases. Two streams flow from the Pool of Active Cases, one comprising those who have become cured and go to the Pool of Previous Cases, and the other comprising those who die of any cause who go to the Pool of Deceased Cases. It is also seen that the Pool of Previous Cases is a reservoir fed by the cured cases and drained by the relapses and deaths.

It is obvious that this model might provide us with important information on the natural history of the disease. It could tell us which persons in the population are prone to develop active disease. It might also answer such questions as who becomes cured in the Pool of Active Cases who remains chronic who relapses These are just some of the few questions which plague the clinician and the epidemiologist, as well as the public health worker

The following simple numerical parameters can be drawn from Fig. 1 the incidence, the prevalence, and the mortality rates. The incidence gives the number of new cases per year in relation to the size of the general population. This rate is composed of the First timers, i.e. a patient who falls ill for the first time, and the Relapses. It is evident that unless there exist rather definite knowledge of

The study presented at the Third International Conference on Sarcoidosis, Stockholm, 1963, was supported by grant No. AI-04817 from the National Institutes of Health, U.S.A.

Prevalence of Pulmonary Sarcoidosis Studied by Mass Chest Radiography

Introduction

Sven Löfgren

The data presented below were based on mass chest radiography. T get some uniformity of the presentations and make comparisons possible the contributors were ad- sed to follow the recommendations given by WHO (Expert Committe on Health Statistics, Technical Report Series No. 164, Geneva 1959). According to these recommendations the frequency figures of an illness can be given either as prevalence or incidence figures.

Prevalence: the frequency of illnesses in existence at any time during a defined period (a year month, a week, etc.) whether they started before or during the period. Alternatively the measurement may be pplied to persons who are ill at any time during the period, whether they became ill before or during the period.

Incidence: the frequency of illnesses commencing during defined period, or of persons becoming ill during the period.

Further the participants were asked to answer special questionnaire, distributed before the meeting (see next page)

pret the rates in a meaningful way The *prevalence* as seen in Fig 1 is the number of active cases who are alive in a population at a given time. This rate will depend not only upon the actual occurrence of the disease and effect of therapy but also on the influence of a series of man-made decisions such as what is considered as an active case what is considered as a cure, etc.—The ordinary *mortality rates* were originally meant to indicate how many persons died *from* the disease. These rates are, however beset with a number of deficiencies—medical, clinical, and administrative in nature. Another and much more objective measure of the lethal effect of the disease, from a public health point of view as well as from the clinical point of view would be a lethality index This index is a comparison between the observed number of deaths and the number of deaths which would have occurred if the patients had the same mortality as the general population.

Even though it may be admitted that studies comprising patients from limited areas might be of great interest, it should be stressed that a complete picture and understanding of the disease is obtained only when the material has a *nationwide* basis. As regards sarcoidosis, no country has been able to obtain the data necessary to fill in the pools and streams described above. The first steps toward such a goal have now been taken in Denmark with the establishment of a nationwide register for all active cases of sarcoidosis, and it is hoped that a continuation of this work will enable us to present a thorough analysis on the problem in the future. It must be foreseen that this will be long-durating, difficult, and expensive.

Miss Penelope Payne is acknowledged for her help in the preparation of the manuscript.

DISCUSSION

DR SILTZBACH I think you will find, Dr Horwitz, that the epidemiologic problem in sarcoidosis will be quantitatively little different from that of tuberculosis. I your four pools, for example. deep pool will be the newly discovered cases of sarcoidosis and those with active disease. An even deeper pool will contain patients who have recovered clinically and are quit well However the active case pool will receive relatively few individuals from the previously activ or recovered group since recurrences in sarcoidosis, unlik tuberculosis, are quite uncommon. The pool of deaths will also be shallow perhaps shallowest of all, since few deaths are recorded as being caused by sarcoidosis. It is the rare pathologist who will find time to do the exhaustive autopsy investigation which is necessary t times, to detect recovered cases of sarcoidosis.

DR ISRAEL Some of the reports today have been of mass surveys with careful clinical follow-up. these provide measure of th prevalence of sarcoidosis t a given point in time. Other reports merely indicat the frequency of typical radiologic changes in mass survey and some reports appear to represent the accumulation of carefully studied cases in clinics over unspecified periods of time These various categories of data are not comparable.

From the Centre of Mass Chest Radiography of the Swedish National Board of Health, Sundbyberg, and St. Göran Hospital, Stockholm, Sweden

International Study of Pulmonary Sarcoidosis in Mass Chest Radiograpy

Hanns J Bauer and Sven Löfgren

In the young history of sarcoidosis not only the unknown etiology but also the epidemiology of the disease are still virgin country for scientific research. Previous studies of the world-wide distribution of sarcoidosis had to rely on single case histories in the literature or on hospital statistics. These individual reports are insufficient for the assessment of the epidemic extension of the disease. First in the era of mass radiography (MR) increasingly used in the examination of large populations during recent decades, it has become possible to carry out prevalence studies. As the radiographic film can disclose only the intrathoracic localisation of the disease, the prevalence statistics are exclusively confined to pulmonary sarcoidosis. On the other hand, they include many asymptomatic cases detected by MR which otherwise would never have been diagnosed. In spite of their limitation, prevalence studies based on reports from MR examinations offer valuable possibilities in estimating the epidemic distribution of pulmonary sarcoidosis and in comparing statistical investigations.

Before this conference was held, a questionnaire was sent to experts on pulmonary sarcoidosis all over the world, asking them to give certain information about the prevalence rates in their countries (see above). We received 30 reports from different parts of the world and were fully aware of the great difficulties entailed in collecting all the statistical data requested. However these data are necessary for a comparative prevalence study on an international basis. The practical purpose for the proceedings of this conference was the compilation of various national statistics revealing principle differences in a short survey.

The results of the international inquiry are presented in the table, in geographical order. Prevalence data from the United States and South America are not included in the table but will be presented separately by Dr Chapman and Dr Purriel, respectively. The prevalence rates of pulmonary sarcoidosis are generally derived from the results of MR surveys carried out once, or periodically on identical population groups. It must be emphasized that only a few of the reports in the table can be considered as representative of nation-wide conditions, as Czechoslovakia, Eire, Finland, Scotland, The Netherlands, Norway, Portugal, Sweden, Switzerland, and New Zealand. The majority of the reports are concerned mostly with prevalence rates obtained from examinations of local or selected populations. Nevertheless, the differences between the prevalence rates reported from various countries or districts thereof are high. Regional statistics from Scotland, Sweden, Switzerland and New Zealand exhibit significant local variations too. Moreover a review of the reports disclosed the evidence of pulmonary sarcoidosis apparently being more often diagnosed in the age groups above 50 years among the Scotch and Nor-

Questionnaire for Prevalence Reports on Sarcoidosis

HANNS J BAUER AND SVEN LÖFGREN

The purpose of this questionnaire is to collect some basic information about the prevalence of sarcoidosis detected by mass chest radiography. The data obtained will be evaluated in a comparative study and the results will be presented as a joint report at the conference. Even incomplete information will be appreciated as a valuable contribution.

1 Number of persons examined	Number of cases of pulmonary sarcoidosis detected by photofluorography	Prevalence of pulmonary sarcoidosis per 100 000 persons examined
Total	Total	Total
Males	Males	Males
Females	Females	Females

If possible, add a detailed distribution of the sarcoidosis cases among the sexes and different age groups (age of 10—19 20—29 30—39 years etc.)

2. a) Have the figures given above been obtained from general surveys of the total adult population, or mass investigations of selected groups?
   b) What was the average attendance in per cent of the population invited to the examination?
   Total % Males Females
   c) Race of the population examined?
   d) Environment of population examined
   Urban? Rural? Mixed?

3. The criteria for establishing the diagnosis of sarcoidosis
   a) Photofluorographic or roentgenological findings only?
   b) Checking of photofluorographic findings by clinical examination, including biopsy?

4. Do you have any experience with respect to the frequency of pulmonary sarcoidosis detected by repeated mass chest radiography of identical population groups in your country during the last ten to fifteen years?
   a) The prevalence of sarcoidosis detected by repeated mass chest radiography of these population groups has
   increased? decreased? remained unchanged?
   b) During the same time the prevalence of pulmonary tuberculosis detected by the same examination has
   increased? decreased? remained unchanged?

wegians than in any of the other countries, where MR statistics indicate maximum prevalence rate in the age groups between 20—40 years in both sexes. — Since the eligible age for MR examinations is usually from about 15 years onward, no prevalence rates could be obtained for children.

The information acquired with regard to the distribution of pulmonary sarcoidosis between the sexes is diverse, and does not indicate any preponderance of either sex. In spite of local variations the reports do not disclose striking differences between prevalence rates of urban and rural populations.

The prevalence rates obtained from MR surveys in Sweden, Norway, Great Britain, Eire and the Netherlands are obviously higher than those reported for other countries. It is, of course, possible that racial or climatic factors might be responsible for the great variations reported. But to some extent the higher rates of prevalence may be due to better recognition and better facilities in diagnosing the disease.

MR surveys are performed mainly with the purpose of pulmonary tuberculosis case finding. In different countries they are carried out on a larger or smaller scale, and the legislative notification systems usually emphasize only case finding of pulmonary tuberculosis. Moreover there is the lack of interest or knowledge of the diverse radiographic patterns which pulmonary sarcoidosis exhibits in its various stages. These circumstances may lead not only to diagnostic misinterpretation but also cause statistical differences of prevalence rates due to the loss of cases. Furthermore, it was evident that the diagnostic criteria and methods were by no means uniform, and that the diagnosis to a large extent was based solely on radiological findings.

As the material presented in the table consists mostly of populations of European descent no significant racial differences could be calculated. On the other hand, the significance of racial factors is evident from the prevalence reports presented from the United States.

A study of the individual reports confirmed the general observation that pulmonary tuberculosis has decreased during recent decades, whereas pulmonary sarcoidosis has increased according to many statistical reports. It is still an open question whether this increase is the consequence of an epidemic development or the product of improved medical knowledge and awareness of the disease combined with the diagnostic facilities offered by the method of mass radiography.

Differences between the prevalence rates of the sexes, various age groups or social categories may be caused entirely by the composition and selection of the population examined. Therefore a careful statistical analysis of the clientele examined is imperative for a proper interpretation of the results of MR examinations and of comparative studies. Furthermore, it must be stressed that the prevalence rates obtained from MR surveys can present only a momentary approximation of the distribution of pulmonary sarcoidosis among a certain population, unless they are repeated at short intervals. According to general experience about 70 per cent of patients with hilar adenopathy present a normal chest film within two years. Therefore MR surveys cannot avoid overlooking a large proportion of patients whose pulmonary sarcoidosis has regressed completely.

The international survey seems to motivate the following requirements for an improved and standardized analysis of the distribution of the disease:

I. Better knowledge of the rather typical radiographic pattern of the disease during the evaluation of MR films.
II. Legislatively stipulated notification of all cases of pulmonary sarcoidosis—especially during MR examinations.
III. Diagnostic standards adaptable for comparative epidemiological studies on an international basis.

Prevalence of Pulmonary Sarcoidosis

Country	Reporter	No. of examined (in thousands)	No. of sarcoidosis cases: Total	Males	Females	Prevalence per 100,000
Scandinavia						
Finland	Patiala	1 430	111			8.1
	Riska & Selroos	155	8			5.1
Norway	Riddervold	1 448	387	181	206	26.7
Sweden	Bauer & Wijkström	I 1,873	1 023	453	570	55
	»	II 1 351	867	396	471	64
Great Britain and Eire						
London	James	868	160	87	73	19
Scotland	Douglas	1 709	141	59	82	8.2 (6.5—18)
N Ireland	Milliken	1 448	149	60	89	10.3
Eire	Logan	383				33.3
European Continent						
Czechoslovakia	Levinský & Altmann	3,436	118	53	65	3.4
France	Turiaf	207	20			c. 10
Germany						
W Berlin	Fried	(2,200)	319	114	205	14.5
Leipzig	Lindig	3 017	134	48	86	13.3
Hungary	Mándi & Kelemen	c. 91	5			5
Italy	Muratore	17	2			(11 6)
The Netherlands	*Orie & Brugge*	4,591	994	370	624	21 6
Poland	Jaroszewicz	93	10			10.7
Portugal	Villar	c. 3,500	6			0.2
Switzerland	Sommer	3 161	515			16.3
Yugoslavia	La Grasta	277	33	6	27	11.9
America						
Canada	Pollak	c. 77	≥8			≥ 10.5
Argentine	Rey	340	17			5.0
	Castelli	695	7			1 0
Brasil	Certain & de P ula	1 810	4			0 2
Uruguay	Purriel	1,839	8			0.4
Asia						
Israel	Rakower	422	7	6	1	1.6
J pan	Hosoda & Nobechi	193	11			5.6
Australia and New Zealand						
Australia	Marshman	1,571	145	66	79	9.2
New Zealand	Reid	1 081	171	88	83	16 (6.1—24.3)

I, II: two surveys   population   university students

TABLE I. The reported cases, distributed according to year of diagnosis

	1961	1962	Total
Sarcoidosis	104	120	224
Sarcoidosis, Observation for	42	46	88
Hilar adenopathy nos	15	24	39
Total	161	190	351

TABLE II. The cases distributed according to roentgenological findings

	Number of patients	Percentage
Hilar adenopathy	140	45
Hilar adenopathy and Pulmonary lesions	91	29
Pulmonary lesions	81	26
Total	312	100

TABLE III. The biopsies distributed according to origin

Origin of biopsy[1]	Number	Percentage
Lung	—	—
Lymph nodes from		
Mediastinum	18	12
Daniels biopsy	95	63
Other	23	15
Liver	—	—
Muscle	2	1
Skin	3	2
Tonsils	1	1
Mucous membrane	3	2
Other	4	3
Not stated	1	1
Total	150	100

[1] If more than one biopsy had been performed in a patient, preference is given according to the order of listing.

reactions as large as 30 mm were occasionally found.

A Kveim test was performed on 26 patients, 16 of whom had a positive result according to criteria worked out by Siltzbach (2). *The number of tested patients is not large*, but the procedure seems to be so promising that efforts will be made to have the test carried out on a larger scale.

According to the notification *forms* 18 patients, or 6 %, had been suffering from erythema nodosum. Presumably this percentage should be taken as an underestimate since such cases occurred in 25 % of a series of patients whose medical records were closely examined[2].

When the number of patients is related to the total population of Denmark, 4.5 million, we find that the annual incidence of new cases of sarcoidosis was 3.4 per 100,000 general population. As the incidence of first time cases of repiratory tuberculosis in the same period was about 20 per 100,000, the incidence of sarcoidosis has been one fifth of the frequency of tuberculosis.

No definite knowledge seems to exist today on whether the morbidity of sarcoidosis is increasing or decreasing. It might therefore be tempting to compare the rates from the previous study Horwitz (1) with those observed in the present study. But, it should be stressed that any comparison between that study and the present one is not justifiable because of the great difference in basic material. Knowledge of the time trend in the incidence will have to wait until further material has been collected in future years.

Rates for the urban and rural population are illustrated in Table IV. The picture presented here is in striking contrast to that for tuberculosis in which the rates are higher in the capital than in the province, and higher in the urban than in the rural population. However, an exact interpretation of this observation is difficult because the rates depend to a great degree upon the routine screening of the population, in terms of both the number of persons examined and also any population shift within the examined group.

For the years 1954—57 a large geographic

From the Danish Tuberculosis Index, Copenhagen, Denmark

Epidemiologic Studies on Sarcoidosis in Denmark Based on a Nation wide Central Register

A Preliminary Report

P H ALSBIRK

A few years ago, the frequent occurrence of sarcoidosis in Denmark was disclosed by a study based on the medical reports which are published annually by the chest clinics, Horwitz (1) Unfortunately these reports were not detailed enough to permit a thorough analysis of the sarcoidosis problem. It was therefore decided to establish nation-wide central registration of all cases of sarcoidosis, with the aim of long range studies on the epidemiology and natural history of the disease. The present paper gives the first results from the material collected in this central register

Since 1961 the chest clinics have reported all first time cases of sarcoidosis to the central register which is located in the Danish Tuberculosis Index. All cases are reported on special notification forms which give detailed identification data and information concerning diagnosis, X-ray findings, etc. According to the notification forms, 351 cases of sarcoidosis were diagnosed at the chest clinics during the years 1961—62 (Collection of material for the present study stopped July 1 1963) The majority of these patients had a diagnosis of either definite or suspicious sarcoidosis, cf. Table I In addition, 39 patients were reported for hilar adenitis, not otherwise specified. Since the latter group of patients did not seem to be systematically reported—reports came from only 10 of the 24 central chest clinics—this group was excluded from the analysis.

Based on information stated on the notification forms, the following account can be given of the clinical findings

There was evidence of roentgenological abnormalities in all patients. In Table II the patients have been divided according to whether these changes were present in the lung fields or hilar regions. In practically all instances, these processes were bilateral.

A biopsy was performed on 150 patients, and epithelioid cell granulomas were demonstrated in three fourths of them. Although the biopsy could be taken from any organ, the vast majority of these were taken from lymph nodes, cf. Table III[1] Daniels biopsy was apparently performed on 95 patients. This number is, however too high because it includes not only patients who were operated on according to the technique indicated by Daniels, but also patients in whom only superficial lymph nodes were removed.

The tuberculin sensitivity cannot be described in detail because the technique dosage, etc. differed, and in one third of the patients, the reaction was stated only as "negative" or positive It should nevertheless be noted that among the patients in whom the exact size of induration was recorded, one half had a reaction of 8 mm or more and

The study presented at the Third International Conference on Sarcoidosis. Stockholm, 1963, was supported by grant No. AI-04817 from the National Institutes of Health, U.S.A.

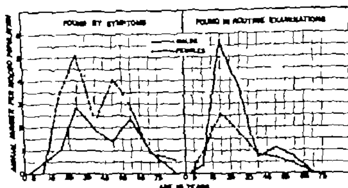


Fig. 2. Incidence of cases found because of symptoms or on routine examinations, respectively by sex and age.

TABLE V. Sarcoidosis patients diagnosed in Præstø County 1953—62, by place of registration

Registered at			
Medical Department	Chest Clinic	Number of patients	Percentage
+	—	12	17
+	+	25	35
—	+	34	48
	Total	71	100

coidosis was diagnosed, but 12, or 17 %, of these cases were registered only at the medical department, cf. Table V. This deficit is rather high, especially considering that there has been traditional close co-operation between the two departments. But it might be even more important that this deficit is composed of a special group. All of these were patients admitted to a medical department because of symptoms, and thus represent the more severe cases of the disease. This fact should be recognized when the natural history of the disease is studied from the present material.

Dr. J. Hårbøl, Chief of the chest clinic of Præstø County and Dr. S. Madsen, Chief of the medical department, County Hospital, Præstø, are thanked for placing the records at our disposal.

References

1. Horwitz, O. Geographic epidemiology of sarcoidosis in Denmark 1954—57. Am. Rev. Resp. Dis., 84: 135, 1961
2. Siltzbach, L. E. Current status of the Nickerson-Kveim reaction. Am. Rev. Resp. Dis., 84: 89, 1961

TABLE IV The incidence of sarcoidosis, by residence

	Annual number	Annual number per 100 000 population
The capital	19.0	2.3
Provincial towns	43.5	3.4
Rural districts	90.0	3.5
Unknown	3.5	
Total	156.0	3.4

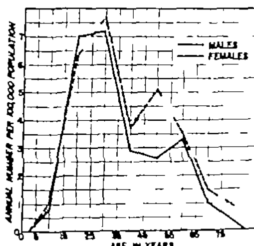


Fig 1 Incidence of sarcoidosis, by sex and age.

area of Jutland showed a rather high frequency of sarcoidosis. The morbidity here was three times greater than that observed in other counties in which the rates were fairly uniform. In the present study this pattern was not repeated, nor was there any systematical geographic variation. The incidence of sarcoidosis was not correlated to the incidence of respiratory tuberculosis by county nor was there any correlation to the geographic pattern of tuberculin sensitivity, occurrence of bovine tuberculosis, or the prevalence of coniferous forests.

The age pattern of sarcoidosis showed a very pronounced peak around the age of 20 years, and a lower and more rounded peak around the age of 50 years, cf. Fig 1 This curve can be broken down into one representing patients found because of symptoms, and one representing those patients found on routine examination. Nearly half of all patients were found because of symptoms, and the age curve for them shows two peaks of approximately the same height, cf. Fig 2. In practically all age groups the highest rates were observed among females. The other half of the patients were diagnosed because of the chest clinics routine screening of the general population, and the age pattern for these cases is shown to the right in Fig 2 This curve is dominated by the youth peak, which is more pronounced among males than among females.

The information on the notification form does not allow a comparison of the severity of the disease among "routine cases" with that among "symptom cases" Off hand, it seems reasonable to assume that the "routine cases" have only vague symptoms, if any since they are diagnosed among working persons.

It would have been of interest to show the age incidence of sarcoidosis among all persons examined at the chest clinic in routine screening this could not be done, as the age composition of this population is not available. However it should be mentioned that the registered frequency of sarcoidosis in this group is much higher than that in the general population. About 800,000 persons are examined yearly by the chest clinics, and among them, 125 cases of sarcoidosis were diagnosed in 1961—62, corresponding to an annual rate of 8 per 100 000 This rate is nearly four times higher than the rate of "symptom cases" in the general population.

The present study is based on reports received from the chest clinics, and a question therefore arises as to how representative is the present material or in other words, how often does it happen that a case is diagnosed without the patient being admitted to a chest clinic? It was impossible to examine this question on a nation-wide basis, but a spot check was done in Presto County comparing the records in the county chest clinic and the medical department of the County Hospital. From 1953 to 1962 a total of 71 cases of sar

From Statens skjermbildefotografering, Oslo, Norway

Sarcoidosis in Norway

Leif Riddervold

umber of cases of pulmonary sarcoidosis, revealed by mass chest radiography during the period 1954—1958, distributed according to age and sex. Absolute figures and per 10,000 persons examined.

Age in years	No. of persons examined	No. of cases	Per 10,000	No. of newly detected cases	Per 10,000
Males					
<15	26,662	—		—	
15—19	63,400	—		—	
20—29	91,505	11	1.20	9	0.98
30—39	137,345	40	2.91	28	2.04
40—49	135,381	47	3.47	17	1.26
50—59	108,703	39	3.59	19	1 75
60—69	76,296	33	4.33	13	1 70
≥ 70	47,933	11	2.29	4	0.83
Total males	687,313	181	2.63	90	1.31
Females					
<15	27,335	—		—	
15—19	68,815	2	0.29	1	0.15
20—29	117 144	15	1.28	11	0.94
30—39	150,706	39	2.59	26	1 73
40—49	145 402	44	3.03	31	2.13
50—59	120,411	57	4.73	32	2 66
60—69	83,002	35	4.22	13	1.57
≥ 70	48,000	14	2.92	5	1.04
Total females	760,835	206	2.71	119	1.56
Total males and females	1 448,148	387	2.67	209	1 44

From the Department of Pulmonary Diseases, University of Helsinki, and Mjölbolsta Hospital, Finland

Sarcoidosis in Finland

J PÄTIÄLÄ, N RIIKA and O SELROOS

In 1960—61 O/Y Yleisröntgen (an institution for mass X-ray examinations) performed 1 450 000 mass X ray examinations in Finland, where the total population (January 1 1961) was 4 477 000 In these series there were 111 roentgenograms where sarcoidosis was suspected Further clinical investigations confirmed the probability of this diagnosis in 60 per cent of the subjects. Estimated prevalence was 4 6/100 000 (JP)

In the tuberculosis district of Raseborg, situated in southern Finland with a population of somewhat less than 210,000 155 672 mass X ray examinations were performed in 1960—1962. In these series 8 cases of sarcoidosis were detected, representing a prevalence of 5 1/100 000 Two of these patients were detected in 1962 However in the same year sarcoidosis was diagnosed in a further 8 subjects from the Raseborg district (NR & OS)

In 1960 the Statistical Department of the Medical Board in Finland registered all diagnoses from every Finnish hospital. Hence it was easy to trace all sarcoidosis patients treated in hospitals during that year The number of cases was 116, representing 97 subjects. (In the same year 11 600 patients were hospitalized for pulmonary tuberculosis.) All the cases were re-evaluated, and we regarded the diagnosis of sarcoidosis as certain in 48 cases, as probable in 23 and as improbable or wrong in 26 cases. On the basis of these figures the prevalence of clinically treated, certain or probable sarcoidosis was 1 6/100 000 inhabitants (NR & OS)

Most of the patients in the mass X ray series had only enlarged hilar glands or slight parenchymal lesions, thus representing the subacute type of sarcoidosis. Among the clinically treated patients one-third (23 out of 69 with intrathoracal, certain or probable sarcoidosis) had severe pulmonary changes.

The above-mentioned prevalence figures for sarcoidosis are markedly lower than those reported from the Scandinavian countries (Sweden, Denmark and Norway) To us this seems to be a factual situation and not an accidental occurrence. The Raseborg district, for example, is one that has been rather thoroughly examined, and there prevalence is only 5.1/100 000 mass X ray examinations during a 3 year period. This figure is in agreement with that based on mass X ray examinations in the whole country during a 2 year period. Real prevalence in the Raseborg district is, however considerably higher as only approximately one-fifth of the cases are diagnosed by mass X ray examination. That the figure for the Raseborg district is nevertheless so low can hardly be explained by insufficient diagnosis other important factors must be involved As far as the whole country is concerned, sarcoidosis seems to be underdiagnosed, to some extent, in Finland but it is improbable that true prevalence exceeds 10/100,000 mass X-ray examinations.

We do not know why there is so much less sarcoidosis in Finland than in the other Scandinavian countries. Perhaps a fact worth noting in this connection is that in Finland pulmonary tuberculosis is even now much more prevalent than in Sweden, Denmark and Norway

TABLE I. Prevalence of pulmonary sarcoidosis in two MR surveys during 1945—1962

Dispensary areas / Prevalence per 100,000	Uppsala	Sörmland	Stockholm County	Norrköping	Östergötland	Skaraborg	Gävleborg	Kalmar	Stockholm City	Västernorrland	Blekinge	Älvsborg	Göteborg-Bohus	Kopparberg	Halland	Göteborg City	Jämtland	Kronoberg	Västmanland	Gävle City	Hälsingborg City	Värmland	Västerbotten	Gotland
Survey I	6	8	—	4	21	—	—	—	44	58	—	44	14	48	28	24	137	21	82	30	23	80	78	—
Survey II	55	63	29	31	—	16	45	26	42	—	72	—	—	—	—		—	—	—	—	—	—	—	29

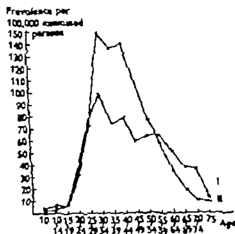


Fig. 1. Prevalence of pulmonary sarcoidosis in different age groups during two M.R. surveys in Sweden (1945—1953 and 1963—1960).

I. M.R. survey average prevalence: 55/100,000 (1,023 cases in 1,873,035 examined persons)

II. M.R. survey average prevalence: 64/100,000 (867 cases in 1,350,560 examined persons)

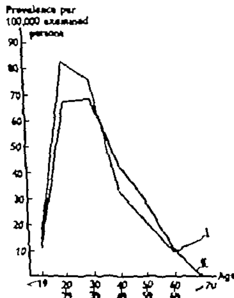


Fig. 2. Prevalence of pulmonary sarcoidosis in different age groups during two M.R. surveys in Stockholm (1949—1952 and 1955—1960).

I. survey 185 cases/423,221 examined, prevalence 44/100,000

II. survey 210 cases/498,121 examined, prevalence 42/100,000

age groups as is shown in fig. 2. Public attendance at both surveys was about 72 per cent of the persons invited. The highest prevalence rates were found between the ages of 25—35 years in both sexes.

The most common radiographic finding was bilateral hilar adenopathy with or without parenchymal lesions. The diverse fibrotic pattern of sarcoid changes clearly predominated in the higher age groups.

The prevalence rates were rather similar at both surveys i.e. 44 and 42 cases per

From the Centre of Mass Chest Radiography of the Royal Swedish Medical Board, Sundbyberg, Sweden

The Prevalence of Pulmonary Sarcoidosis in Swedish Mass Radiography Surveys

H J BAUER and S WIJKSTRÖM

From the start of Mass Radiography surveys (MR) on a nation wide scale in 1945 the statistical reports dealt not only with pathological, intrathoracic diseases of tuberculous but also of non-tuberculous etiology Among the latter group the cases of pulmonary sarcoidosis (p.s.) aroused special interest, as this condition had previously been encountered rather seldom, but was found quite frequently in general surveys of the population. Since 1952 all cases of p.s., detected by the surveys carried out by the Centre of Mass Radiography in the various parts of the country have been registered separately in the annual statistics. Thus it became possible to obtain certain information on the approximate sex and age distribution of the disease in various districts of Sweden.

The following study presents the prevalence rates of p.s. derived from two MR surveys during the periods 1945—1953 and 1953—1960 when the population of several counties and cities were examined twice. The public response varied from 70 to 98 per cent of the persons invited. The officially stipulated age for attendance is from ten years.

The MR films were evaluated by a special staff of readers at the Centre. The positive findings together with the films were sent to the respective local dispensaries or chest clinics for medical examination and follow up. The final diagnoses were then reported to the Centre.

During the first survey 1 023 cases of p.s. were detected among 1 873 055 persons examined, i.e. 55 cases per 100 000 examined. During the second survey starting from 1953, the prevalence rate of p.s. rose to 64 cases per 100,000 persons examined, i.e. 867 cases among 1,350,560 persons. It is quite probable that the increased prevalence figures of the second survey are due to a better knowledge and awareness of the readers and the medical diagnosticians.

The prevalence rates reported from different dispensary areas varied considerably In fact, they ranged from 4 to 137 cases per 100,000 persons examined, as is shown in table I It becomes also obvious that p.s. was more frequently diagnosed during the second survey of the same dispensary areas. Compared with the international survey of p.s. prevalence rates, the Swedish local figures indicate similar variations. There seems to be no plausible explanation other than the possible existence of local endemic conditions, or the human factor

Fig 1 illustrates the age distribution of p.s. in both surveys. The highest rates were found between the ages of 20—40 years in both sexes, reaching a maximum between 30—35 years. Females showed slightly higher prevalence rates than males.

The results of two MR surveys carried out in the City of Stockholm reflect an analogous distribution among the sexes and different

From the Royal Northern Hospital, London, Great Britain

Prevalence of Intrathoracic Sarcoidosis in Britain

D. G. James and G. Z. Brett

The prevalence of suspected sarcoidosis, detected by mass radiography surveys, is approximately 20 per 100,000 population in England and Wales. Although the overall prevalence is similar in men and women, it is twice as common (39 per 100,000) in women of the child-bearing years of life. It is extremely common in Irish women (200 per 100,000) and Irish men (120 per 100,000) X-rayed in London.

For the future it is clear that ariety of techniques must be used to detect the disease and assess its overall distribution. Mass radiography surveys will undoubtedly continue to uncover the bulk of suspected disease. It is desirable that histological confirmation be sought in such cases— by scalene lymph node biopsy aspiration liver biopsy or by means of the Kveim test. At the same time, it is hoped that those responsible for such survey will routinely collect and store sera from these patients. Such sera may eventually provide important retrospective evidence, if the aetiological agent is discovered and a serological test developed. There are certain obscure clinical groups in which the storing of sera will be a worthwhile investment they include erythema nodosum, uveitis, hypercalciuria, hyperglobulinaemia and patients exhibiting persistently negativ Mantoux status following B.C.G. vaccination.

TABLE I. Mass radiography rates of suspected sarcoidosis compared with activ respiratory tuberculosis, per 100,000 persons examined in England and Wales during 1955—60 (figures kindly supplied by General Register Office)

Rate per 100,000	1955	1956	1957	1958	1959	1960
Sarcoidosis	10	12	14	20	17	20
Tuberculosis	220	190	180	190	180	160

100 000 persons examined. These figures are distinctly below the average rates reported for the whole of the country. This may indicate a different frequency of the disease in the urban and in the rural population.

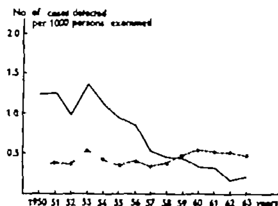


Fig. 3. The prevalence of pulmonary sarcoidosis and active pulmonary tuberculosis detected by MR examinations of the Swedish Armed Forces

— — — pulm. sarcoidosis

——— newly detected active pulm. tb

The statistics based on the annual routine examinations of the Swedish Armed Forces during the years 1950—1963 show an increasing prevalence of p.s. compared with the decreasing frequency of newly detected pulmonary tuberculosis (see fig. 3). The majority of the annually varying population examined, consisted largely of male recruits between the ages of 18—25 years. But also staff personnel within and above this age group are included. Since 1960 it became quite evident that p.s. was being more often diagnosed than active pulmonary tuberculosis.

Just as the prevalence rates vary between the different dispensary areas so do the diagnostic criteria and methods. From the individual dispensary reports, however, it appears that biopsy has been increasingly performed during recent years. But a large part of the diagnoses relied on clinical criteria, viz., positive radiographic findings combined with low or negative tuberculin sensitivity and the absence of subjective ailments.

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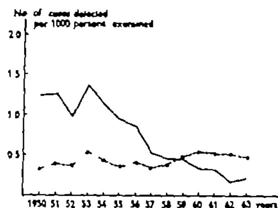


Fig. 3. *The prevalence of pulmonary sarcoidosis and active pulmonary tuberculosis detected by MR examinations of the Swedish Armed Forces.*

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Answers to questionnaire for sarcoidosis prevalence

(cf. corresponding tables 1, 2, and 4)

1. Number of persons examined		Number of pulmonary sarcoidosis detected by photofluorography		Prevalence of pulmonary sarcoidosis per 100,000 persons examined	
Total	867,637	Total	160	Total	19
Males	533,421	Males	87	Males	16
Females	334,216	Females	73	Females	22

For age and sex distribution, se table 1.

2.) Race of the population examined mixed but predominantly English, see table 2 c.

   d) Environment of population examined mixed, see table 2 d.

3. The criteria for establishing the diagnosis of sarcoidosis photofluorographic or roentgenological findings only

4.) The prevalence of sarcoidosis detected by repeated mass chest radiography of these population groups has increased.

   b) During the same time the prevalence of pulmonary tuberculosis detected by the same examination has decreased, see table 4.

Questionnaire. TABLE 1 Prevalence rates of suspected sarcoidosis by age and sex, discovered by Mass Radiography in the North West Metropolitan Region, 1957—60.

Age group (years)	Male (M) or Female (F)	Number surveyed	Suspected sarcoidosis: No. of Cases	Suspected sarcoidosis: Rate/100,000
14—24	M	97,815	15	13
25—44	M	255,248	59	22
45 +	M	180,358	15	8
14—24	F	113,687	10	9
25—44	F	132,561	51	39
45 +	F	85,968	12	14
Total	M	533,421	87	16
Total	F	334,216	73	22

Questionnaire. TABLE 2 c. Prevalence rates of sarcoidosis and active pulmonary tuberculosis discovered by static London Mass Radiography Unit during 1957—60 subdivided according to birth place*

Birthplace	Male (M) or Female (F)	Number surveyed	Prevalence rate/100,000: Sarcoidosis	Prevalence rate/100,000: Tuberculosis
Britain	M	40,782	50	600
Britain	F	38,416	20	350
Eire	M	3,373	120	2,100
Eire	F	4,547	200	1,300

50 % of the examinees were patients referred by General Practitioners.

Questionnaire. TABLE 2 d. Prevalence rates of suspected sarcoidosis and active tuberculosis discovered by mass radiography during 1957—60 in the various sectors of the North West Metropolitan Region

Sector	Number surveyed	Prevalence rate/100,000: Sarcoidosis	Prevalence rate/100,000: Tuberculosis
Central London	234,877	12	190
North London	110,715	36	550
Middlesex	222,795	13	130
Hertfordshire + Bedfordshire	299,250	21	81
Total	867,637	19	170

Questionnaire. TABLE 4. Frequency of pulmonary sarcoidosis and pulmonary tuberculosis detected by mass chest radiography in England and Wales 1955—1960

Rate for 100,000	1955	1956	1957	1958	1959	1960
Sarcoidosis	10	12	14	20	17	20
Tuberculosis	220	190	180	190	180	160

Table II Prevalence rates of suspected sarcoidosis and active tuberculosis discovered by mass radiography during 1958 (Dr G Z. Brett)

Region	Number surveyed	Prevalense rates per 100 000 Sarcoidosis	Tuberculosis
North West Metropolitan	214,288	20	190
South East Metropolitan	138,870	10	270
Birmingham	338,362	30	300
Wales	180,433	20	180
England and Wales	3,323,910	20	190

Table III Prevalence rates of suspected sarcoidosis by age and sex, discovered by mass radiography in the North West Metropolitan Region 1957—60 (Dr G Z. Brett)

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14—24	M	97,815	13	13
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Total	M	533 421	87	16
Total	F	334,216	73	22

Table IV Prevalence rates of sarcoidosis and active pulmonary tuberculosis discovered by a static London Mass Radiography Unit during 1957—60 subdivided according to birth place (50 % of the examinees were patients referred by General Practitioners) (Dr G Z. Brett)

Birthplace	Male (M) or Female (F)	Number surveyed	Prevalence rate/100,000 Sarcoidosis	Tuberculosis
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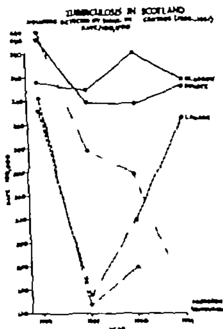


Fig. 2

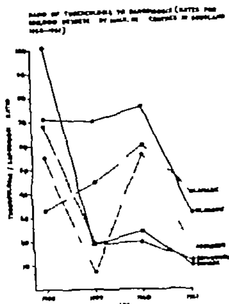


Fig. 3

was relegated from the realms of clinical curiosity to the relatively commonplace.

The problem of the prevalence of sarcoidosis as judged from M.M.R. statistics is, therefore, complex, an attempt to compare it with tuberculosis from M.M.R. returns is even more so. Here comparison is being made between condition which is uncommon and in which the criteria for diagnosis have not altered in the years under review and one which is common and in which the criteria for diagnosis are changing. Trends in tuberculous morbidity indicated by M.M.R. figures can, in large measure, be explained by a changed outlook on the part of the chest physician. Macgregor (1962) has shown that *changing concepts of chemotherapy and* chemoprophylaxis have affected the assessment standards of chest physicians as far as tuberculosis is concerned. In M.M.R. surveys in Scotland from 1953 onwards the proportion of active to significant tuberculosis (the latter being defined as the sum of cases regarded as active or requiring observation) increased from 26.9 in 1953 — 56 to 63.4 % in 1959—60, indicating a decreasing inclination to diagnose inactive tuberculosis. The interpretation of M.M.R. statistics as far as tuberculosis is concerned has, therefore, been changing over the years. The chest physician has been more prone to diagnose disease requiring treatment and which has therefore been labelled active. This has affected the whole trend of the M.M.R. figures for tuberculosis and is likely to have maintained the notification rates at an artificially high level. It largely explains some of the apparent anomalies in Fig. 2.

If sarcoidosis and tuberculosis were aetiologically related one might expect the prevalence rates to move together and ratio of *one to the other to be relatively constant.* There is no such constancy in the ratio tuberculosis/sarcoidosis from M.M.R. findings and the overall impression is that there is no association between the trends of prevalence of sarcoidosis and tuberculosis as determined by the diagnosis produced by mass radiography of the general public (Fig. 3)

From the Department of Respiratory Diseases, University of Edinburgh, Great Britain

Epidemiology of Sarcoidosis in Scotland

A. C. Douglas

The most accurate assessment of the prevalence of a condition which can be *detected* radiographically is likely to be obtained from mass surveys of the general population. In Scotland mass campaigns were conducted in 1957 and 1958, embracing large numbers of the population (84 4 % in Edinburgh) and the figures available for sarcoid prevalence then probably reflect quite closely the true state of affairs in the community 1958 was the year when the greatest M.M.R. coverage of the population was made and in that year the prevalence rate for sarcoidosis in the five main centres in Scotland (comprising urban and rural population in varying proportions) varied from 5 to 12 per 100 000 With regard to assessing trends of prevalence from M M.R. statistics, figures prior to 1958 are incomplete and unreliable for sarcoidosis and I have concerned myself only with th years 1958 to 1961 (the latest year for which full details of M.M.R. activities are available) Since 1958 attention has been increasingly focused on those groups of the population which are subject to higher than average risk from tuberculosis so that comparisons between the prevalence rate for sarcoidosis in the years subsequent to 1958 and the 1958 figure are not strictly valid. Trends in succeeding years have been erratic but the impression is that there may be a general increase in the rate (to 8 to 14 for 4 of the centres and to 34 for the 5th) One must remember however that these rates are calculated from small numbers and small increases mean large "swings" in the graph (Fig 1) e.g the high rate of 41 per 100,000 for Aberdeen in 1959 refers to 11 cases out of under 30,000

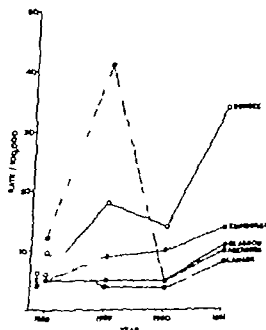


Fig 1

There is little doubt that increasing awareness of sarcoidosis in recent years has resulted in the diagnosis being made more frequently and this, of course, affects M M R. returns which for statistical purposes record the number of cases confirmed by subsequent clinical examination. In Edinburgh, for example, prior to 1952, sarcoidosis was a seeming rarity After 1952, however sarcoidosis was increasingly recognised, with the result that some 200 cases were registered over th next 10 years and the condition

ase is predominantly male disease as far as pick-up from M.M.R. is concerned. Also the age distribution of tuberculous morbidity has been changing over the past decade, but there is nothing in the Edinburgh series to suggest that this has been the case with sarcoidosis. This, however would apply more to males where the peak morbidity has moved rapidly into the older adult age group than to females where the peak of incidence has remained much the same in the younger adult age group.

Finally in our personal series an attempt was made by questionnaire to determine if any relationships existed between occupation, social class, the patient' geographical location since birth onwards, and the incidence of sarcoidosis. There was no discernible predilection of sarcoidosis for any social group or for any part of the City of Edinburgh, and occupations aried from chartered accountant to chicken sexer from barrister to barman.

I am extremely grateful to Mr W. R. Robertson, Statistical Branch, Scottish Home and Health Department, for his help in compiling the M.M.R. data, and to Dr Ian M. Macgregor Senior Medical Officer Department of Health for Scotland, for his continued interest, and invaluable help in the preparation of this paper

Prevalence Rates for Sarcoidosis in the British Armed Forces 1958—1961

Through the courtesy of the Director General of Medical Services I ha been able to obtain the prevalence rates for sarcoidosis in the British Army Navy and Air Force over the years 1958—1961 These figures refer of course, almost entirely to young adult males.

In the Army the prevalence ranged from 3 to 7 per 100,000 (Table I)

In the Navy relatively higher prevalence rate in the 20' was consistently recorded over the years (Table II) This higher rate may simply reflect the greater use of routine Mass Radiography in this Service.

In the Royal Air Force rates aried from 7.8 to 16.9 (Table III) and tuberculosis/sarcoidosis ratios fluctuated in manner which did not suggest *much of* correlation in the prevalence of the two diseases (Table IV)

TABLE I Sarcoidosis prevalence in British Army 1958—1961 (Rates/100,000)

Year	Age groups 15—24	25—34	35—44	Total
1958	1	13	—	4
1959	2	8	—	3
1960	5	—	15	5
1961	6	9	—	7

TABLE II. Sarcoidosis prevalence in Royal Navy 1958—1961 (Rates/100,000)

Year	Rate per 100,000 (Male)	Rate per 100,000 (Female)
1958	24	—
1959	25	—
1960	20	116
1961	21	—

TABLE III. Sarcoidosis prevalence in Royal Air Force 1958—1961 (rates/100,000)

Year	Age groups < 20 yrs.	20—24	25—30	30—34	35—39	40—44	Total
1958	3.5	2.1	4.7	28.5	18.3	41.6	7.8
1959	12.4	18.1	13.6	34.6	24.7	—	16.9
1960	3.9	17.2	4.1	13.6	—	—	11.4
1961	3.7	10.5	33.5	14.3	8.1	—	12.7

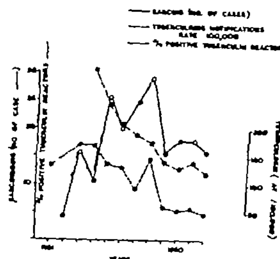


Fig. 4

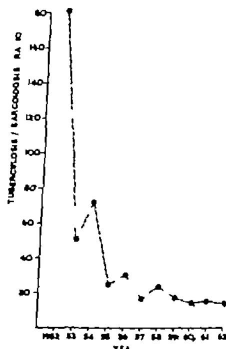


Fig. 5

I have looked at this problem in another way. In this composite graph (Fig. 4) in which I have freely taken liberties with scales, the trends of tuberculosis morbidity in Edinburgh are shown by the percentage of positive tuberculin reactors in school leavers and the notification rates for tuberculosis. Superimposed are the actual numbers of cases of sarcoidosis registered by the Chest Units in Edinburgh over the decade 1952/1962. Initially when interest was only developing, the figure was naturally small (only 4 in 1952 from a population of around 1/2 million) but by 1955 it is probably true to say that most cases of recognised sarcoidosis developing in Edinburgh would be registered because of the awareness by other physicians of our interest. From 1955 then, one could fairly make comparison of sarcoidosis and tuberculosis from the same catchment area by employing the figures for sarcoidosis in our personal series and the notification rates for tuberculosis. When this is expressed as a ratio (Fig. 5) there is an initial precipitous fall which is explained by the very small number of cases of sarcoidosis registered in 1952. The ratio falls from 24.5 in 1955 (when the tuberculosis notification rate was 136 per 100,000) to 13.9 in 1962 (when the tuberculosis notification rate was 47 per 100,000). Although there is variation in the ratio, this is not sufficiently marked, particularly in the years in the wake of the 1958 M.M.R. campaign, to allow an emphatic statement that there is no aetiological relationship as judged by this method of assessment.

There is, however, evidence of lack of correlation between tuberculosis and sarcoidosis in the sex incidence. Most series have indicated that sarcoidosis is predominantly a female disease and in Edinburgh the female to male ratio for all cases is around 4 to 1. This sex difference is borne out by the M.M.R. figures from the various centres, although the disparity in these is not so marked as in the Edinburgh series. The Scottish National Statistics for Tuberculosis indicate a male predominance of tuberculosis and tubercul-

From South Tyrone Hospital, Dungannon, Co. Tyrone, N. Ireland

Sarcoidosis in Northern Ireland

T. G. MILLIKEN

Answers to Questionnaire on Sarcoidosis Prevalence

1. Number of persons examined—refer to table I. I have separate figures for males and females examined only up to the year 1957, see tables II and III, and I thought you might also be interested in table IV.

2. a. The figures given are the result mainly of general surveys of the adult population on a voluntary basis. Two major

TABLE I. Pulmonary tuberculosis and sarcoidosis 1945—62: mass radiography

		Pulmonary Tuberculosis				Sarcoidosis	
Year	Population X-rayed	Active	Per 100,000	Inactive	Per 100,000	Total	Per 100,000
1945	16,275	124	760	314	1929	3	18.4
1946	30,173	203	672	573	1902	6	19.8
1947	30,036	164	546	523	1747	5	16.6
1948	32,123	162	535	351	1092	2	6.2
1949	24,349	158	643	576	2346	0	0
1950	54,250	167	307	644	1188	5	9.2
1951	64,587	328	507	812	1257	1	1.5
1952	74,383	454	610	758	1019	8	10.7
1953	67,977	395	580	715	1060	6	8.9
1954	96,239	517	537	1014	1053	5	5.2
1955	101,709	437	429	1021	1003	13	12.7
1956	124,459	331	266	1030	827	10	8.0
1957	117,541	273	232	1295	1102	10	8.5
1958	114,804	221	192	1209	1055	21	18.3
1959	136,628	227	168	1480	1083	22	16.1
1960	119,284	211	177	1178	983	14	11.7
1961	139,393	127	91	705	503	11	7.9
1962	104,378	79	76	603	577	7	6.7

TABLE IV Prevalence of tuberculosis and sarcoidosis (rates/100,000) and tuberculosis/sarcoidosis ratios in R.A.F personnel 1958—1961

Year	Age groups									
	< 20	20—24	25—29	30—34	35—39	40—44	45—49	50—54	< 55	Total
1958										
Tuberculosis rate	182	82	61	66	67	93	0	576	0	90
Sarcoidosis rate	5.5	2.1	4 7	28.5	18.3	41 6	0	0	0	7.8
Tuberculosis/sarcoidosis	33 1	38.8	1.3	2.3	3 7	2.2				11.5
1959										
Tuberculosis rate	81	89	2	43	4	62	0	146	0	71
Sarcoidosis rate	13.4	18.1	13 6	34 6	24 7	0	0	0	0	16.9
Tuberculosis/sarcoidosis	6 0	4.9	2 0	1.2	3.0					4.2
1960										
Tuberculosis rate	3.	93	58	94	69	82	128	0	1951	81
Sarcoidosis rate	5.9	17..	4 1	15 6	0	0	0	0	0	11 4
Tuberculosis/sarcoidosis	5.9	5 4	14 1	6.0						7 1
1961										
Tuberculosis rat	69	45	33	86	49	24	0	72	0	47
Sarcoidosis rat	5 7	10 5	33 5	14 3	8.1	0	0	0	0	12.7
Tuberculosis/sarcoidosis	12 1	4.3	1 0	6 0	6 0					3.7

TABLE IV. Cases of sarcoidosis found during the period 1945—62. Radiographic characteristics related to age and sex groups

Age Groups	Hilar		Infiltrative		Miliary		Hilar and Infiltrative		Hilar and Miliary		Infiltrates and Miliary		Totals	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0—14	0	0	0	1	0	0	0	0	0	0	0	0	0	1
15—24	9	12	4	4	1	1	2	3	0	0	0	1	16	21
25—34	8	16	8	8	4	3	3	3	0	0	0	1	23	31
35—44	5	6	7	8	0	1	2	2	1	0	0	2	15	19
45—59	1	3	1	5	1	0	1	3	0	1	1	3	5	15
≥ 60	0	0	1	2	0	0	0	0	0	0	0	0	1	
	23	37	21	28	6	5	8	11	1	1	1	7	60	89
Totals		60		49		11		19		2		8		149

diagnosed in the last 10 years in population of 132,000, 90 % of which have been confirmed histologically These latter figures are, *of course*, unusual in that we have been specially interested in sarcoidosis during the last eight years and have invited all practitioners to refer cases of erythema nodosum to us. The results of the observations on these cases will form the basis for our paper Nevertheless, it would appear that there is higher incidence of sarcoidosis in the County of Tyrone where we work, which is typical rural area with population of 134,000

Acknowledgements My thanks are due to Dr J Ritchie, Director of Mass Radiography for N. Ireland, who kindly supplied the M. R. figures, and to colleagues who replied to my questionnaire.

TABLE II. Cases of pulmonary sarcoidosis 1945—62 inclusive, classified by sex and year

Year	Sarcoidosis Male	Female	Total
1945	2	1	3
1946	3	3	6
1947	2	3	5
1948	1	1	2
1949	0	0	0
1950	2	3	5
1951	1	0	1
1952	4	4	8
1953	2	4	6
1954	3	2	5
1955	7	6	13
1956	2	8	10
1957	3	7	10
1958	7	14	21
1959	8	14	22
1960	6	8	14
1961	3	8	11
1962	4	3	7
Total	60	89	149

TABLE III Cases of pulmonary sarcoidosis found from 1945—62 inclusive classified by age and sex

Age Group	Sarcoidosis Male	Female	Total
0—14	0	1	1
15—24	16	21	37
25—34	23	31	54
35—44	15	19	34
45—59	5	15	20
60	1	2	3
Total	60	89	149

(urban) and a number of minor (rural) mass investigations of selected groups were carried out, but the figures from these would not account for more than one tenth of the total. These took place between 1957 and 1961

b The average attendance of the population invited to the examination would be about 35 % for both males and females, but no exact figure is available.

c. Race white.

d. Urban one third, rural one third, mixed one third.

3 a. and b. All of the cases discovered by Mass Radiography were checked also by clinical examination, but biopsy was rarely carried out. Exact figures are not available.

4 a. and b. It could be said that roughly the same population has been examined on a voluntary basis by repeated mass chest radiography every three years for the last 15 years, at least from about 1953 onwards. This was part of a drive to eliminate tuberculosis, which was very successful.

The incidence of new cases of tuberculosis detected in 1962 is exactly one tenth that of 1945. There does not appear to be a parallel decline in the incidence of sarcoidosis, but more interest was taken in the latter diagnosis in the last eight years. This point does not apply so much to the Mass Radiography findings as to the findings obtained from my clinical colleagues, which follow

I have made an attempt during the last few months to get some idea of the prevalence of sarcoidosis diagnosed by my colleagues throughout the North of Ireland, apart from those diagnosed by Mass Radiography I have had replies from only about half so far but submit the following figures *in the essentially* urban district of Belfast there were some 90 cases in the last 10 years, only about 20 of which were confirmed by histology This covers a population of about 700,000, 500 000 of whom are within the city of Belfast. The proportion of replies from the urban physicians is the same as that from the rural area, i.e. 60

My country colleagues in the rural areas report 94 cases from about the same population, i. e. 700 000, 30 of which were confirmed histologically In addition to these figures for rural areas, I must add the figures for our own district where about 170 cases have been

From the 1st Clinic for Tuberculosis, Faculty of General Medicine, The Charles IV University
Prague, Czechoslovakia

Prevalence of Pulmonary Sarcoidosis in Czechoslovakia

L. Levinský and V. Altmann

We attempted to collect the available data on the prevalence of pulmonary sarcoidosis in Czechoslovakia. The country is divided into 11 regions and 118 districts and has nearly 14 million inhabitants. We requested all phthisiologists who are conducting the campaign against tuberculosis in their respective districts, all regional and district hospitals, and all sanatoria for the treatment of tuberculosis to collaborate. With their help we obtained the following data

1 The prevalence of pulmonary sarcoidosis detected by mass X-ray surveys of persons over 14 years of age, in 63 districts. The total number of subjects examined was 3,436,992. The number of cases of pulmonary sarcoidosis ascertained by this method was 118 the prevalence 3.4 per 100,000 persons examined. In the individual regions from 0 to 7.9, and in the individual districts from 0 to 20.7 per 100,000.

2. The prevalence of pulmonary sarcoidosis according to the number of persons registered as suffering from this disease in district outpatient departments. Patients with pulmonary sarcoidosis are registered in 79 out of 118 districts. Their population is 9,009,965. There were 393 registered cases of



Fig 1 Prevalence of pulmonary sarcoidosis in the individual regions of Czechoslovakia per 100,000 inhabitants.

In the shaded regions the number of patients hospitalized exceeded that of patients registered in outpatient departments. In the unshaded regions the situation was the reverse.

From Peamount Hospital, Newcastle, Co. Dublin, Eire

Prevalence of Sarcoidosis in the Irish Republic

JOSEPH LOGAN

1 From a total of 383 000 M.M.R. examinations in Dublin in 1958, 1959, 1960 and 1962, there was an overall discovery of 33 cases of sarcoidosis per 100 000 examinations.

2 A preponderance of 5 females to 1 male found in 1958 was not found in later years.

3 During these years there was no apparent increase in the prevalence.
In 135 biopsy-confirmed cases of sarcoidosis the maximum age-incidence was in the 10—20 age groups (33 %) and 76 under 40 years old.
There was no difference between the number of males and females.

Discovery of Respiratory Tuberculosis and Sarcoidosis by Mass Radiography in the Irish Republic

Rates per 100,000 examinations

	Respiratory Tuberculosis	Sarcoidosis
1958	229	30.6
1959	261	20.5
1960	?	42.0
1962	?	39.0

Note

1 Many of these patients were referred by their own doctor because of symptoms. The number is unknown.

2 The figures refer to the Dublin area only (predominantly urban).

3 It has not been found possible to check the diagnosis of sarcoidosis for the years 1958–1960. The figures appear to refer to X Ray appearances suggestive of sarcoidosis.

From Hôpital Bichat, Paris, Hôpital Sainte-Eugénie, Lyon, and Hôpital Boucicaut, Paris, France

Epidemiologic Investigation on Sarcoidosis in France

J Turiaf J Brun and André Meyer

The results of this investigation are from various regions of the French territory

I — Compared frequency of tuberculosis and sarcoidosis based on mass x-rays of groups —

Students 97,799

Tuberculosis	208—212 per 100,000
Sarcoidosis	6—6,1 per 100,000

Others 514 493

Tuberculosis	1,073—208 per 100,000
Sarcoidosis	52—10,1 per 100,000

Total number of examined individuals 612,292

Tuberculosis	1,281—211 per 100,000
Sarcoidosis	58—9.5 per 100,000

Pulmonary Sarcoidosis in France According to Sex, Age, Profession and Domicile

Familial sarcoidosis one case per 268 — mother and daughter

Frequency according to sex

Men	160—59.7 %
Women	108—40.3 %
Total	268

Frequency according to age and sex

Age	Men	Women
10—19 years — 16 cases	7— 2.6 %	9— 3.4 %
20—29 years — 105 cases	77—28.7 %	28—10.5 %
30—39 years — 90 cases	54—20.1 %	36—13.4 %
≥ 40 years 57 cases	22— 8.2 %	35—13.1 %
Total	160—59.6 %	108—40.4 %

Frequency according to age

Age	Number of Patients	
10—19 years	16	6 %
20—29 years	105	39 %
30—39 years	90	34 %
≥ 40 years	57	21 %
Total	268	

Of special interest are the definitely higher percentages in men aged 20 to 40 years and women over 40.

Frequency according to profession

Intellectual and sedentary professions. Students. Office workers. Store keepers. Housekeepers	149—56 %
Labourers. Factory workers and miners	89—33 %
Other occupations and no profession	30—11 %

Frequency according to domicile

Urban	209—78 %
Rural	31—11.5 %
Mixed	28—10.5 %

Summary

1. In France, according to the mass X-rays, sarcoidosis occurs approximately in 9 to 10 individuals per 100,000 examined.

2. Among examined individuals with sarcoidosis approximately 60 % were men and 40 % women. According to the age the higher percentage was between 20 and 40 years in men, over 40 years in women — 33 % of the patients were labourers. — 78 % live in cities — 11.5 % in rural areas and 10.5 have mixed domicile.

pulmonary sarcoidosis representing a prevalence of 4.3 per 100 000 inhabitants from 2.9 to 6.5 in the individual regions.

3 We obtained reports from 132 medical and phthisiological hospital departments and sanatoria out of 278 (this is less than half) on the number of subjects treated, as in-patients, for pulmonary sarcoidosis. The total number of hospitalized patients was 426 and was thus even higher than the number of patients registered in outpatient departments. The prevalence of pulmonary sarcoidosis detected in this manner varied from 0 6 to 9 6 per 100 000 inhabitants in the individual regions.

4 In figure 1 the shaded areas show regions in which the number of patients with pulmonary sarcoidosis treated in hospitals and sanatoria exceeded the number of those registered in outpatient departments. In 6 unshaded regions the situation was the reverse. Taking all cases, including those reported on from both hospitals and outpatient departments, we obtained 490 known cases of pulmonary sarcoidosis. This shows that the actual prevalence of pulmonary sarcoidosis in Czechoslovakia is much higher than that estimated on the basis of the number of cases currently registered in the outpatient departments. We estimate that prevalence is somewhat more than 10 per 100,000 inhabitants.

It is quite clear that in Czechoslovakia the differences in the prevalence figures for pulmonary sarcoidosis in the different districts and regions are due to the fact that the physician responsible for the highest figure has a special interest in, and a better knowledge of, sarcoidosis. But the fact that erythema nodosum is less frequent in this country than, for example, in Scandinavia, indicates that a certain geographical difference in the forms of sarcoidosis can exist between the Scandinavian and the central european countries.

In this country the ratio of women to men is 1.5 to 1 The largest number of cases is found in the age group between 30 and 39 years. In 10 percent of the cases pulmonary sarcoidosis was diagnosed only from the roentgen-films in 90 percent it was verified by a clinical examination, and in 30 percent by biopsy

c) Deutschstämmig (mitteleuropäisch)

d) Gemischte Bevölkerung.

3. a) Jeder Schirmbildbefund wurde durch Großaufnahme überprüft.

b) Die meisten Befunde wurden durch klinische Beobachtung geklärt, wobei die Scalenus-Biopsie in allen Fällen routinemäßig zur Anwendung kam.

4. a) Zahlen können für einen Zeitraum von 10—15 Jahren nicht vorgelegt werden. Es ist unter den hiesigen Verhältnissen außerdem schwer zu entscheiden, ob die immer häufiger gestellte Diagnose „Morbus Boeck" auf einer echten Zunahme der Erkrankungen oder nur auf einer besseren Kenntnis des Krankheitsbildes beruht. Vielleicht kann einen Anhalt die Tatsache geben, daß in den letzten Jahren, gemessen an dem klinischen Krankengut, männliche Patienten mit einem Morbus Boeck viel öfter beobachtet worden sind als in früheren Jahren, in denen sich der Morbus Boeck fast ausschließlich auf Frauen beschränkte.

Außerdem sind in letzter Zeit hin und wieder Boeck-Erkrankungen mit akutem Beginn, etwa im Sinne des Löfgren-Syndroms, beobachtet worden, die früher zu den Raritäten zählten.

Da alle Schirmbilder in unserem Lande archiviert werden — auch die Schirmbilder ohne Befund — haben wir durch Schirmbild-Direktvergleiche oft passagere bilaterale Adenopathien feststellen können, die uns früher in diesem Ausmaß nicht bekannt waren.

b) Hierüber geben die Tabellen III und IV Auskunft. Aus ihnen ist zu ersehen, daß im Berichtszeitraum das Verhältnis zwischen Morbus Boeck und aktiver Lungentuberkulose sich immer mehr zu Gunsten des Morbus Boeck verschoben hat.

Im allgemeinen sind für die aktiven Lungentuberkulosen sowohl die Bestandszahlen als auch die Zahlen der Neuzugänge, ähnlich wie in anderen Ländern, zurückgegangen. Die Ursachen des im Jahr 1962 festzustellenden Stillstandes sind noch nicht aufgeklärt. Möglicherweise ist er nicht epidemiologischer Natur, sondern darauf zurückzuführen, daß sich bei uns immer mehr der Standpunkt durchgesetzt hat: Jeder bei der jährlichen Aufstellung des Katasters neuentdeckte Befund (Schirmbildvergleiche!) ist behandlungsbedürftig, mithin als aktiv anzusehen.

TABELLE III Durch Volksröntgenreihenuntersuchungen im *Bezirk Leipzig* wurden neu entdeckt an aktiver Lungentbc. und Lungensarkoidose

Jahr	Anzahl der untersuchten Personen	Personen mit aktiver Lungentuberkulose			Personen mit Lungensarkoidose			Verhältnis von *Lungensarkoidose* zu Lungentbc.	Prozentuale Beteiligung
		männl.	weibl.	Sa.	männl.	weibl.	Sa.		
1960	1 025 718	550	323	873				1 33	83,6
auf				83 1	4	21	25		
100 000							2 4		
1961	971 905	480	280	760	23	29	52	1 : 15	82,8
auf 100 000				78,2			5,3		
1962	1 018 935	485	280	765	1	36	57	1 13	84,9
auf 100 000				75 1			5,6		

TABELLE IV Bestand und Neuzugänge an aktiver Lungentbc. und Lungen-Boeck im Bezirk Leipzig bezogen auf 100 000 Einwohner

Jahr	Bestand am Ende des Berichtsjahres			Neuzugänge			Wohnbevölkerung		
	männl.	weibl.	Sa	männl.	weibl.	Sa.	männl.	weibl.	Sa.
1960									
Tbk.	1 342 1	603 9	925 7	152,5	74 8	108,_	675 884	843 140	1 519 024
Boeck	10 7	14,3	12,7	2,8	5 1	4 1			
B T	1 125	1 42	1 72	1 54	1 14 6	1 26			
1961									
Tbk.	1 292,9	581 7	898,6	129,2	63 1	92 3	672 477	837 134	1 509 611
Boeck	15,5	16,7	17,3	5 0	5,3	5 1			
B T	1 83	1 : 31	1 52	1 26	1 12	1 18			
1962									
Tbk.	1 289 1	577 4	895 1	153 5	59 6	92 6	675 680	838 069	1 513 749
Boeck	19 9	25,5	23 0	6,4	6,8	6,6			
B T	1 64	1 23	1 39	1 20	1 9	1 14			

2 a) Die Angaben stammen aus allgemeinen Untersuchungen der gesamten Bevölkerung (Aufstellung des Volksröntgenkatasters) für 1960 und 1961 vom 10 Lebensjahr ab, für 1962 vom 12 Lebensjahr ab.

b) Siehe Tabelle III Eine Aufgliederung nach Geschlechtern wurde nicht vorgenommen. Es ist aber anzunehmen, daß die prozentuale Beteiligung bei Männern und Frauen ziemlich gleichmäßig war

) Deutschstämmig (mitteleuropäisch)

d) Gemischte Bevölkerung.

3.) Jeder Schirmbildbefund wurde durch *Großaufnahme überprüft.*

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Tabelle III. *Durch Volksröntgenreihenuntersuchungen im Bezirk Leipzig wurden neu entdeckt an aktiver Lungentbc. und Lungensarkoidose*

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auf				85,1	4	21	25		
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Jahr	Bestand am Ende des Berichtsjahres			Neuzugänge			Wohnbevölkerung		
	männl.	weibl.	Sa.	männl.	weibl.	Sa.	männl.	weibl.	Sa.
1960									
Tbk.	1 342,1	605,9	925,7	152,5	74,8	108,5	675 884	843 140	1 519 024
Boeck	10,7	14,3	12,7	2,8	5,1	4,1			
B : T	1 : 125	1 : 42	1 : 72	1 : 54	1 : 14,6	1 : 26			
1961									
Tbk.	1 292,9	581,7	898,6	129,2	63,1	92,5	672 477	837 134	1 509 611
Boeck	15,5	18,7	17,3	5,0	5,3	5,1			
B : T	1 : 83	1 : 31	1 : 52	1 : 26	1 : 12	1 : 18			
1962									
Tbk.	1 289,1	577,4	895,1	133,5	59,6	92,6	675 680	838 069	1 513 749
Boeck	19,9	25,5	23,0	6,4	6,8	6,6			
B : T	1 : 64	1 : 23	1 : 39	1 : 20	1 : 9	1 : 14			

2. a) Die Angaben stammen aus allgemeinen Untersuchungen der gesamten Bevölkerung (Aufstellung des Volksröntgenkatasters) für 1960 und 1961 vom 10. Lebensjahr ab, für 1962 vom 12. Lebensjahr ab.

b) Siehe Tabelle III. Eine Aufgliederung nach Geschlechtern wurde nicht vorgenommen. Es ist aber anzunehmen, daß die prozentual Beteiligung bei Männern und Frauen ziemlich gleichmäßig war.

From the Department of Tuberculosis and Pathological Institute of the University Medical School, Debrecen, Hungary

Sarcoidosis in Eastern-Hungary

László Mándi and János Kelemen

The problem of sarcoidosis has scarcely been dealt with in Hungarian medical literature. This disease has been studied by us more intensively since 1956. Up to the middle of 1963 we succeeded in obtaining data on 86 cases of sarcoidosis in Eastern Hungary. The majority of these patients came from 2 counties with a population of about one million.

Among these 86 patients there were *82 cases of thoracic disease* and 4 of peripheral lymph node sarcoidosis. Consequently thoracic sarcoidosis is not a rare disease even in Hungary.

We cannot present an accurate epidemiological study of Hungary. Formerly screening examinations were also carried out here, but only in certain age-groups, in factories and in the military forces. Mass-survey of the whole population was not introduced until the last 2—3 years. In our town (Debrecen) of 150,000 inhabitants such surveys have been made since 1958. At first, however only about 70 percent of the population presented themselves for examination. Exact and comprehensive screening dates from last year. Here, according to our findings, there are about 1—3 cases of sarcoidosis a year per 100,000 inhabitants. In other parts of Hungary this number is quite uncertain, since this disease was hardly ever dealt with.

More than half of our cases of pulmonary sarcoidosis were screened because of various complaints (pain in the chest, difficult breathing, cough, subfebrility etc.) and were detected by these symptoms. Erythema nodosum, as the tracer of sarcoidosis, formed a different group and 14 such cases were observed.

More than one-third of the cases were revealed by mass chest radiography. One case was found in autopsy material.

Three cases of sarcoidosis of the peripheral lymph nodes on the neck and one in the axilla were diagnosed by biopsy of the lymph nodes, which appeared as tumour-like enlargements.

It is stated in the literature that, as a rule, sarcoidosis is most frequent between the ages of 25—35 years and is rarely observed in childhood. In our material, the age group 15—25 years is predominant. It is striking that in *15 cases we observed sarcoidosis in childhood.*

According to the literature the ratio between men and women is 34 : 48.

As regards the residential distribution of our patients, 45 lived in towns and 37 in the country. The higher number of the townsmen can probably be accounted for by the greater possibilities these had of medical supervision. Exact data will be available only when all our villagers have undergone screening for a number of years.

Our patients occupational distribution was: family members 28, university students 21, industrial workers 12, intellectuals 7, chauffeurs 4, agriculturists 3 and others 8. Consequently occupational analyses did not furnish any useful factor from an etiological point of view.

There are no pine-woods in the part of the country examined. Among grasses, however we have found two well-known plants of frequent occurrence—*Typha latifolia* and *angustifolia*—from the *Ziehl Neelsen positive pollen* of which *wax-like material* can be obtained by treating with alcohol-ether.

From Queen Frederika Hospital, Piraeus-Athens, Greece

Sarcoidosis in Greece

A. Kordosis and P Lazarou

Sarcoidosis in Greece is very rare, probably because of its geographic mediterranean location, where sarcoidosis is not frequent. The number of published cases is very small (21 cases) (Quoted from A. Kordosis and P Lazarou Erythema Nodosum, Bilateral Hilar Lymphoma and Arthralgia. Lofgren's Syndrome. Sarcoidosis. The Review Medical Annals Vol. 2 No. 5—6. Sept.—Dec. 1963, p. 453—464 Athens—Greece.)

From Algemeen Provinciaal-, Stads- en Academisch Ziekenhuis, Groningen, The Netherlands

Prevalence of Pulmonary Sarcoidosis in The Netherlands

N. G. M. Orie and R. ter Brugge

Netherlands total count with Amsterdam, Den Haag, Rotterdam and Utrecht

Area	Number of persons examined			Sarcoidosis		
	Males	Females	Total	Males	Females	Total
Netherlands	1,630,574	2,062 435	3,693,009	335	567	902 (24.5)
Amsterdam	113,195	159,080	272,275	7	15	22
Den Haag	86,863	136,225	223,088	11	18	29
Rotterdam	144,803	196,298	341 101	12	14	26
Utrecht	35,569	36,335	71,904	5	10	15
Total Netherlands	2,011,006	2,580,371	4,591,377	370 (18.4)	624 (24.2)	994 (21.6)

Answers to questionnaire on sarcoidosis prevalence.

1. Data of latest population survey in the Netherlands (4—4 1/2 years duration) Number of fresh cases of sarcoidosis detected per 100,000 population.

The data cover little less than half of the population of the Netherlands (per 1. 1. 1963 11,889,962. 16 years and older 8,200,001)

2.) Population mass survey
   b) The average attendance for those who were expected to come 90—95 %.
   The following groups were not expected:
   children < 16 years,
   people attending regular examinations (railway personnel),
   people attending regular dispensary control for tuberculosis.
) Race Dutch
3. The large majority of the cases has been confirmed by biopsy and/or clinical examinations.
4. The data on tuberculosis and sarcoidosis found in mass population survey can best be answered by giving the data of the following sample.

Mass population survey of the Northern Netherlands
(Ter Brugge)

Year	Activ. pulm. tuberculosis	Sarcoidosis
1952	226	68
1953	192	68
1954	168	59
1955	140	76
1956	112	83
1957	104	106
1958	75	96
1959	77	69
1960	63	83
1961	54	84
1962	33	84

From Ospedale Civile, Lecce, Italy

Notes on Sarcoidosis in Puglia and Lucania, Italy 1952—1961

F Muratore

As yet, we have no statistical data in Italy on the prevalence of sarcoidosis. Our own researches are confined to cases observed in Puglia and Lucania between 1952 and 1961 (Puglia population 3,500 000 area 19 346 sq km. Lucania population 665 000 area 9987 sq km)

The cases referred to here were selected from those whose clinical criteria were supported by the results of biopsy 26 other cases were discarded because, though biopsy was positive, corresponding clinical criteria were lacking Other cases were discarded, despite the presence of the suspected clinical features, because they were not confirmed by biopsy tests.

For obtaining our statistical data we utilized the reports issued by several Institutes, Clinics, Hospitals in Puglia and Lucania Anatomical Pathology Institute of Bari University Medical Clinic and Medical Pathology Institutes of Bari University Lecce's Hospital, Taranto, Brindisi Foggia, Potenza and Matera Hospitals, and other hospitals of the two provinces.

The number of cases collected by us must, undoubtedly be considered only as an approximat assessment. Nevertheless, they represent the first attempt to present statistics on the distribution of the disease in these two Italian provinces.

For each of our case reports we have given year of observation, sex, age occupation, place of birth, and any thoracic and lymphoid involvement, other organs frequently affected, and the presence of dysproteinemia.

Examination of our cases showed that

1 there is a remarkable predominance of female subjects.
2. at the X-ray examination the hilar pulmonary involvement was positive in 56 7 % negative in 22 % and dubious in 7.2 % In 11.3 % the chest X-ray examination was not performed.
3. There was hepatic involvement in 42.3 % and a hepatosplenomegaly in 6.2 %
4 We did not find the occupational data of any particular interest.

On the basis of the results of our investigation it may be stated that In Puglia, during a period of 10 years, there have been 89 (2.5 per 100 000 inhabitants) cases of sarcoidosis.

In Lucania, during the same period only 8 cases (1.2 per 100 000 inhabitants) were observed. Such a great difference in the number of cases in the two bordering provinces is due entirely to the fact that in Puglia we are more aware of the disease, and therefore look more thoroughly for it.

Although sarcoidosis exists both in Puglia and in Lucania it must be considered an uncommon disease. In fact, out of 10 435 X-ray examinations performed during recent years at the X-ray Department of the Hospital in Lecce sarcoidosis was found only in two cases while in 6,728 photofluorographic examinations made during 1961 and 1962 at th Tuberculosis Dispensary Clinic there was not a single case with the slightest sign or suspicion of sarcoidosis.

Reference

Cummings, M. M. Dunner E., Schmidt R. H. Barnwell, J R.: Concepts of epidemiology of Sarcoidosis. Postgrad. Med. 19 467 1956.

From Algemeen Provinciaal- Stads- en Academisch Ziekenhuis, Groningen, The Netherlands

Prevalence of Pulmonary Sarcoidosis in The Netherlands

N. G. M. Orie and R. ter Brugge

Netherlands total count with Amsterdam, Den Haag, Rotterdam and Utrecht

Area	Number of persons examined			Sarcoidosis		
	Males	Females	Total	Males	Females	Total
Netherlands	1,630,574	2,062,433	3,683,009	335	567	902 (24.5)
Amsterdam	113,195	159,080	272,275	7	15	22
Den Haag	86,863	136,225	223,088	11	18	29
Rotterdam	144,805	196,296	341 101	12	14	26
Utrecht	33,569	38,335	71,904	3	10	13
Total Netherlands	2,011,006	2,580,371	4,[illegible]91,377	370 (18.4)	624 (24.2)	994 (21.6)

Answers to questionnaire on sarcoidosis prev alence.

1. Data of latest population survey in the Netherlands (4—4 1/2 years duration) Number of fresh cases of sarcoidosis de tected per 100,000 population.

The data cover little less than half of the population of the Netherlands (per 1. 1. 1963 11,889,962 16 years and older 8,200,501)

2.) Population mass survey
   b) The erage attendance for those who were expected to come 90—95 .
   The following groups were not ex pected
   children < 16 years,
   people attending regular examinations railway personnel)
   people attending regular dispensary control for tuberculosis.
) Race Dutch
3. The large majority of the cases has been confirmed by biopsy and/or clinical ex aminations.
4. The data on tuberculosis and sarcoidosis found in a mass population survey can best be answered by giving the data of the following sample.

Mass population survey of the Northern Netherlands
(Ter Brugge)

Year	Active pulm. tuberculosis	Sarcoidosis
1952	226	68
1953	192	68
1954	180	59
1955	140	76
1956	112	85
1957	104	106
1958	75	96
1959	77	69
1960	63	83
1961	54	84
1962	33	84

From Ospedale Civile, Lecce, Italy

Notes on Sarcoidosis in Puglia and Lucania, Italy 1952—1961

F Muratore

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Reference

Cummings, M M. Dunner E., Schmidt, R. H Barnwell, J R.. Concepts of epidemiology of Sarcoidosis. Postgrad. Med. *19* 467 1956.

TABLE III. Sarcoidosis among patients treated at the Tuberculosis Institute in Warsaw

Year	Total number of patients	Number of cases	Males	Females	Urban	Rural
1950	537	3	1	2	2	1
1951	543	1		1		1
1952	490	1		1	1	
1953	401	3	2	1	1	2
1954	398	3	3		1	2
1955	395					
1956	383	1		1		1
1957	356	3	3		1	2
1958	291	6	2	4	6	
1959	292	2	1	1		2
1960	605	3	1	2	1	2
1961	621	4	1	3	1	3
1962	602	6	4	2	2	4
Total	5 704	36	18	18	16	20
Sarcoidal tissue reaction to Tb and aspergillosis		2				
Sarcoidosis suspected but not confirmed		9				

TABLE IV. Age distribution of patients

Age	10—19	20—29	30—39	40—49	50—59	60—69	Total
Number	7	15	7	6	0	1	36

2. Patients with clinical symptoms who were hospitalized in order to establish proper diagnosis.
3. Patients, with previous false diagnosis of tuberculosis, who did not improve after chemotherapy; this suggested the possibility of sarcoidosis.

The data are summarized in Table II.

In 75 percent of the cases the diagnosis was based on the histopathologic examinations, mainly of lymph-nodes specimens obtained by scalene node biopsy. In the remaining 25 percent of the cases this diagnosis was made on the basis of the syndrome of clinical symptoms, as well as on radiological examination and tuberculin tests.

Table III presents more detailed data on the patients with BBS treated at the Tuberculosis Institute.

Summary

BBS in Poland is rather infrequently diagnosed. Orientational data, obtained from mass chest radiophotography of university students, imply that this disease occurs less frequently in Poland than in other countries which report their medical statistics.

From the Instytut Gruzlicy Warszawa, Poland

Tentative Data Concerning the Frequency of Pulmonary Sarcoidosis in Poland

Wiwa Jaroszewicz

In Poland sarcoidosis (BBS) is a disease which occurs rather seldom. We cannot say at the present time, whether there is really a low frequency of BBS or that this is due to insufficient detection of the disease.

Mass chest radiophotography is directed mainly to tuberculosis detection, because of the requirements of the epidemiological situation in Poland. These examinations are carried out systematically. They concern mainly selected population groups, which are chosen according to the aspect of epidemiological tuberculosis control.

The most detailed examinations are made of recruits and university students, the latter being also tuberculin tested. These examinations may serve as a basis for estimating the frequency of BBS. They cannot be regarded however as representative of the sarcoidosis frequency in the whole population.

The number of detected cases is so small that it is difficult to describe this as incidence" during a year. Table I shows the total number of registered patients. The final diagnosis of BBS was usually made in a hospital.

The cases from chest clinics and hospitals might serve as another source of information on sarcoidosis frequency in Poland.

In such institutions sarcoidosis may be diagnosed in the following groups of persons:

I. Patients without *clinical symptoms* in whom the lesions in the lungs were detected accidentally, that is, either by mass chest radiophotography or by other control examinations.

Table I. Sarcoidosis detected among university students by mass chest radiophotography

University Academic Center	Years (academic)	Total number of persons examined	Number of sarcoidosis cases
Warsaw	1960/61	22,965	1
	1961/62	23,512	3
	1962/63	25,985	1
Total		72,457	5
Zabrze	1952—1962	5,584	4
Gliwice	*1957—1962*	*15,000*	1

Table II. Sarcoidosis in patients treated at Medical Academy Hospitals of Tuberculosis and Lung Diseases.

Centre	Years	Total number of patients	Number of sarcoidosis cases
1. Zabrze	1958—1962	2,500	30
2. Łódź	1958—1962	6,260	13
3. Szczecin	*1958—1962*		*22*
4. Kraków	1958—1962	3,091	13
5. Wrocław	1958—1962	1,961	12
6. Institut of Tuberculosis in Warsaw	1950—1962	5 701	36

TABLE I show the changes in the laboratory data available

Tests	Total No. of pts. studied	Normal	Slightly elevated	Elevated	Considerably elevated	Lowered
Sedimentation rate	19	12	7			
Blood calcium	19	9	8		2	
Blood phosphorus	7	6		1		
Alcaline phosphatase	9	5		4		
Total proteins	18	7		11		
Blood albumen	18	11				7
Blood globulin	18	4			14	

TABLE II show the results in relation to the various stages of the disease

Stages of the disease	Number of cases	Normal	Lowered *albumin*	Elevated α_2 glob.	Elevated β glob.	Elevated γ glob.
Stage I	2	2				
Stage II	6	4	1			2
Stage III	4	—	4	1	1	4

ment. Two patients with bilateral hilar lymphadenitis cleared up completely one spontaneously the other on cortisone. Five patients with bilateral adenitis and mottling were given corticotherapy four improved and one remained stationary. Of three patients with extensive, bilateral, pulmonary lesions, one cleared up on vitamin D therapy and 2 got progressively worse under corticotherapy. One of the latter died of respiratory insufficiency.

From the Lisboa University Hospital Portugal

Sarcoidosis in Portugal

T G VILLAR

Sarcoidosis seems to be a relatively rare condition in Portugal, and the first case was only published in 1938

To determine the prevalence of sarcoidosis in Portugal we sent out a circular letter questionnaire to all institutions and physicians that might have recognised these cases. Besides this we undertook a careful review of the Portuguese literature on sarcoidosis.

83 of the circulars were answered but only 45 cases could be identified from 1929 to the present time. Only in 34 cases was the data available sufficient for a positive diagnosis. 17 of these cases were personally diagnosed by the author Most of the other cases were reported from one of the three University towns. An average of 3 new cases have been recognised in the last 7 years.

Of the 34 cases on which we have complete data, 18 were males and 16 females. The youngest patient was 15 years old and the eldest 68.

In 9 of these patients the sarcoid changes were apparently localised to one organ or system — skin lungs 4 lymph nodes 3 In all the other cases lesions involved 2 or more structures — lungs 22, lymph nodes 21 skin 14 bones 9 eyes 3 liver 3 and larynx 1

15 patients had no symptoms 7 complained of general symptoms respiratory symptoms were present in 10 patients and deficient vision in 2 skin lesions were found in 14 patients. Erythema nodosum was only reported in one case 7 years before sarcoidosis was diagnosed. Enlarged superficial lymph-glands were found in 26 patients. The spleen was enlarged in one patient and the liver in 5— liver function tests were positive in one and biopsy was positive in 3. Tuberculin tests were reported in 31 cases, all except 3 of which were repeatedly negative. One gave a positive von Pirquet test and 2 a slightly positive 1 Mantoux test.

The Kveim test was made in 7 patients, using antigen furnished by Dr Siltzbach in 1955 It was positive in 4 cases, negative in one, and 2 patients did not come back for the reading

Electrophoresis of the serum proteins was performed in 12 patients, and table II shows the results in relation to the various stages of the disease.

The most frequent electrophoretic pattern was a lowered albumin and a marked elevation of the γ globulin fraction. The patient with elevated a_2 and β globulins had an associated non-specific infection.

Sputum and/or sarcoid tissue inoculated in the guinea pig in 27 cases was uniformly negative.

We classified the chest X-rays of 34 patients as follows read as negative 4 hilar lymphadenitis (one unilateral) 6 B.H L. + mottling 15 bilateral reticulo-nodular shadows 4 pulmonary fibrosis 3 and segmental condensations 2 Bronchography in 4 patients, was normal in one, showed narrowed bronchi with terminal dilatations in 2 and the right apical lower lobe bronchus obstructed by sarcoid tissue in 1

X ray of the bones of the hands and/or feet was performed in 25 patients and osteitis cystica" was found in 10 cases.

A reasonable follow-up was possible in 12 patients. Two who only had skin lesions, improved slowly under prednisone treat

From the Hospital for tuberculosis and pulmonary diseases Jordanovac in Zagreb, University of Zagreb, Yugoslavia

A Report on the Epidemiological Situation of Sarcoidosis in Yugoslavia

Milivoj La Grasta

According to the recommendations of the Swedish Committee on Sarcoidosis, I should like to give a short report on the epidemiological situation of sarcoidosis in Yugoslavia.

Obviously prevalence data are of most importance in assessing the frequency of pulmonary sarcoidosis and in comparing frequency figures for different parts of the world. Such prevalence data can be obtained only from mass X-ray examinations of large population groups.

In Yugoslavia, mass chest radiography (fluorography) has been performed during recent years not only in the larger cities and industrial centres, but also in some rural areas. However the primary purpose of these fluorographic examinations was to detect pulmonary tuberculosis and only secondarily the other diseases of the chest. Sarcoidosis was not specifically registered as a special disease, in the course of these fluorographies. Consequently I am unable to present a prevalence report on sarcoidosis in Yugoslavia.

However in one of the six republics comprising the federal republic of Yugoslavia,

Table I Fluorographic incidence of pulmonary sarcoidosis in 1961 in Slovenia, Yugoslavia/according to Forus' data

Sex	Age groups									Total
	0—9	10—19	20—29	30—39	40—49	50—59	60—69	70—79	80—	
Number of fluorographically examined inhabitants										
Total	1	7,087	69,301	62,715	43,118	47,630	30,572	13,490	3,102	277,016
Males	—	4,147	35,444	29,917	19,833	22,502	13,250	5,547	1 479	132,119
Females	1	2,940	33,857	32,798	23,285	25,128	17,322	7,943	1,623	144,897
Number of cases of sarcoidosis										
Total	—	—	11	12	2	6	1	1	—	33
Males	—	—	1	2	—	1	1	1	—	6
Females	—	—	10	10	2	5	—	—	—	27
Per 100,000 fluorographically examined inhabitants										
Total	—	—	15.8	19.1	4.6	12.5	3 2	7 4	—	11.9
Males	—	—	2.8	6.6	—	4 4	7.5	18.0	—	4.5
Females	—	—	29.5	30.4	8.5	19.8	—	—	—	18.6

Aus dem Sanatorium Braunwald, Schweiz

Prevalenz der pulmonalen Sarkoidose in der Schweiz

Erwin Sommer

Wir fanden in der Schweiz unter 3 160.000 Schirmbildaufnahmen 515 Sarkoidosen, das sind 16 auf 100 000 Untersuchte. Analog fanden Bauer und Wijkström in Schweden unter 3.224 000 Schirmbildern 4 mal mehr Sarkoidosen, nämlich 1900 d. i. 60 auf 100 000 Untersuchungen. Dieser grosse Unterschied beruht sicher nicht nur auf der intensiveren Fahndung in Schweden, sondern spricht zweifellos für ein wesentlich häufigeres Vorkommen der Sarkoidose in Schweden als in der Schweiz. Wie wir aus den Statistiken von Löfgren entnehmen können, ist dieses vermehrte Vorkommen der Sarkoidose in Schweden zu einem großen Teil durch die zahlreichen Fälle von akutem BHL-Syndrom mit Erythema nodosum (Löfgren) bedingt. Diese Fälle verbessern in Schweden wegen ihrer relativen Gutartigkeit wesentlich die durchschnittliche Prognose aller Sarkoidosefälle, was in Schweden ganz offensichtlich zu einer optimistischeren Beurteilung der Sarkoidose führt als in einigen anderen Ländern. In der Schweiz beobachten wir vor allem die subakuten, subchronischen und chronischen Verlaufsformen ähnlich wie in den USA, wovon sich Löfgren persönlich in amerikanischen Kliniken überzeugen konnte. Durch diese verschiedenartige Zusammensetzung des Sarkoidose-Materials von Land zu Land ist somit auch die Prognose in den verschiedenen Ländern eine andere und in der Schweiz ganz offensichtlich etwas schlechter als in Schweden. Nach unserer Erfahrung beträgt die Mortalität an Sarkoidose und deren unmittelbaren Folgen in der Schweiz 3—5 %

From the Royal Edward Laurentian Hospital, Montreal, Canada

Epidemiology of Sarcoidosis in Canada

B. Pollak

The figures on the prevalence of sarcoidosis in Canada, which we have been able to obtain, are based on the results of three mass X-ray surveys done in 1960 and 1961 in British Columbia and Ontario. The results are listed in the report compiled by Dr Bauer. They show a prevalence of 10.5 per hundred thousand persons X-rayed.

There have been, of course, many more surveys, but all of them were conducted for the detection of tuberculosis. In most cases, only the total number of non-tuberculous conditions was reported without any further classification.

In recent years, more mass tuberculin surveys have been done and only the positive reactors were X-rayed. Such surveys, of course, lose their significance for prevalence studies of sarcoidosis or other non-tuberculous lung conditions.

Not included in the above-given figures were surveys conducted among the Eskimoes and Indians. These sections of our population were surveyed thoroughly and repeatedly because of the very high tuberculosis incidence among them. From the Eastern Arctic region, all Eskimoes found to have abnormal chest X-rays are evacuated to the Mountain Sanatorium in Hamilton for further diagnostic studies. Among 1,200 such cases investigated during the past twelve years, not one case of sarcoidosis has been found. Reports from the Department of National Health and Welfare, Foothills Region, indicate that, in 1961 among the total Indian and Eskimo population of thirty-one thousand, one hundred and eighty-three (31 183) twenty thousand, three hundred and thirteen (20,313) X-rays were taken without discovery of a single case of sarcoidosis. It is interesting to note that the Indians of the Province of Alberta had a tuberculosis morbidity rate of 375 per hundred thousand compared to the rest of the population of the province of 24 per hundred thousand. The Eskimoes had five times higher tuberculosis morbidity rate than the Alberta Indians. It seems worthwhile noting that, among the Negro population of the United States, not only the tuberculous rate is higher than in the white population, but, also, that sarcoidosis is found more frequently.

The only three known cases of sarcoidosis among Indians were diagnosed at the Coqualeetza Indian Hospital, in Sardis, British Columbia in 1962.

To summarize the prevalence of sarcoidosis in Canada is 10.5 per hundred thousand persons X-rayed. This result was obtained only from a small number of surveys, because in the majority no exact results on non-tuberculous conditions could be obtained.

An almost complete absence of sarcoidosis among Eskimoes and Indians was noted.

TABLE II Cases of sarcoidosis established and treated in 21 special hospitals and clinics in Yugoslavia showing sex and age distribution

Number of cases of sarcoidosis

Sex	Age groups									Total
	0—9	10—19	20—29	30—39	40—49	50—59	60—69	70—79	80—	
Total	—	9	62	59	38	29	9	1	—	207
Males	—	6	22	20	15	5	1	1	—	70
Females	—	3	40	39	23	24	8	—	—	137

TABLE III Localization of sarcoidosis in these cases

Pulmonary forms		Extrapulmonary forms		Combination of pulmonary and extrapulm. forms		Total	
Number	%	Number	%	Number	%	Number	
136	66	33	16	38	18	207	100

the republic of Slovenia, 277 016 persons were fluorographically examined in 1961 In the course of these fluorographic examinations, pulmonary sarcoidosis was registered specifically The fluorographic incidence of pulmonary sarcoidosis for persons examined in Slovenia is shown in table I

I have tried to obtain an idea of the general epidemiological situation of sarcoidosis in Yugoslavia in another way I sent a questionnaire to 26 special hospitals and clinics in Yugoslavia. All patients where sarcoidosis was detected by X ray or clinical examinations, were very probably sent to one or other of these hospitals or clinics to establish the diagnosis of sarcoidosis or for treatment. I have received the required data, from 21 out of these 26 hospitals or clinics, on a total of 207 established cases of sarcoidosis and 7 suspected cases. The sex and age distribution of these cases and the localization of sarcoidosis are shown in tables II and III.

Diagnosis of sarcoidosis was established also histologically in 117 cases or 56 and by autopsy in only 3 cases. Tuberculin tests were made in 173 patients and were negative in 82 Approximately 75 of the patients were from urban districts.

Conclusion

Fluorographic incidence of pulmonary sarcoidosis in Slovenia in 1961 and the analysis of data on 207 cases of sarcoidosis received from 21 special hospitals and clinics in Yugoslavia showed that sarcoidosis is not such a rare disease in Yugoslavia.

expected on the basis of chance. Such observations bear out observations with respect to twins noted by Gilg.

Another interesting development in American studies of sarcoidosis is the delineation of small pockets of what appear to be excessive prevalence. First observed and noted in Veterans Administration study that focused attention on county in the state of Washington and small village in Georgia, areas of peculiarly high prevalence ha been noted by others. Kendig (10) reports such situation in Nansemond County Virginia, and two adjacent counties, where the principal agricultural crop is peanuts. Hammarsten (11) has noted similar aggregation in sparsely populated county of southeastern Oklahoma, and Comstock and his colleagues described like concentration in Muscogee County Georgia. Less well localized with respect to area is Dickie observation (12) of exceptional numbers of cases among students at the University of Wisconsin.

In summary it may be said that, on the basis of work up to the present, ecological factors in sarcoidosis seem to include broadly the Southeast, the Great Lakes area and New England an ethnic basis rural background with perhaps significant association with farm animals or pine forests trend toward familial frequency and perhaps peculiarly local pocketing that may be independent of kinship

The association of serological studies in patients with sarcoidosis with high prevalence of tuberculin-type allergy to unclassified mycobacteria among their household contacts — if these observations are correct — makes perhaps worthwhile to consider comparativ epidemiology of unclassified mycobacteria with such epidemiological information as exists with respect to sarcoidosis. The map of geographic prevalence of sensitivity to PPD-B (15) in Navy recruits presents similarities to the distribution of sarcoidosis as seen in the map of birthplaces of U S. Army cases, which seems to resemble the table given by Gundelfinger and Britton. This distribution is quite unlike that shown for the distribution of PPD-S reactors (14)

PPD-B, of course, like any other mycobacterial antigen, has certain element of nonspecificity as pointed out by Nissen-Meyer (15) This substance may elicit responses from individuals sensitized by other strains of unclassified mycobacteria and in the opinion of some authorities (16, 17) it is closely related to avian tubercle bacilli.

Unfortunately figures for highly localized areas of either sarcoidosis or sensitivity to unclassified mycobacterial antigens are too sparse for extended comment, but some parallelism can be seen between the map published by Shook et al. (8) for sarcoidosis in Oklahoma and the distribution maps of PPD-B reactors.

Comparatively little is vailable on tuberculin reactivity of dairy herds in the United States, but maps of reactors for Texas seem to indicate concentrations of reacti animals in areas where the prevalence of sarcoidosis is apparently high. The studies of Mallman and Mallman (19, 20) indicate that much of this tuberculin reactivity is associated with small lesions from which they have been able to culture unclassified mycobacteria.

A recent report from our laboratory shows that unclassified mycobacteria can be cultured from 50 of 50 ml. samples of raw milk as it reaches pasteurization plants (21) Many of these organisms, of course, are of little interest, but apparently good examples of each of Runyon groups has been recovered. Further Scammon et al. (22) has recently reported that the classic method of pasteurization does not destroy all unclassified mycobacteria, an observation that has also been made independently in our laboratory

The implication is not necessarily that dairy cattle excrete unclassified organisms in their milk, but rather that contamination from the premises, even in well-maintained dairy barns, is quite possible. Other animals, swine, chickens, possibly rats or mice might also serv to contaminate premises of milk herds. The point serves to reinforce the point of view expressed by the Baltimore epidemiological study that exposure to farm animals" may be factor of some importance in the ecology of sarcoidosis.

It is obviously not necessary to insist upon

From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas

Epidemiology of Sarcoidosis in the United States of America

JOHN S CHAPMAN

Studies of sarcoidosis in the United States have been strongly influenced by ethnic and geographic factors. The high prevalence among Negroes as opposed to Caucasians, estimated variously from 6 to 1 to as high as 10—12 to 1 is an outstanding feature of the disease in the United States. Beginning with the study made by the Veterans Administration and by the Army and the Navy geographic distribution has colored the approach of nearly all students of sarcoidosis. While Carr and Gage have dissented on this point, their cases necessarily are drawn from a special group and probably are less representative of the true picture of geographic distribution of disease than are the groups reported from the Armed Forces—Veterans Administration. Evidence from these studies points to special areas in the Southeast, in New England and in the northern Midwest.

Preponderance of sarcoidosis in the Southeast may possibly reflect a high proportion of Negroes in the population. But other factors may be involved and some of these have been studied. Comstock and his associates particularly investigated pica among the rural Negroes in Georgia (1) Gentry et al undertook examination of soil types, with attention to beryllium content, and of rural as opposed to urban residence. Dunner (2) Cummings (3) and their associates, impressed by the prevalence of pine woods, have made various correlations with the distribution of special types of forest, but have also considered rainfall and soil types. Others (4 5) have investigated the capacity of pine pollen to produce granuloma. The excellent epidemiological study of the Johns Hopkins group (6—8) revealed a rural background as one of the most outstanding features of patients with sarcoidosis as opposed to carefully matched controls. Not only had more sarcoidosis patients originated from a rural background, but they had lived in rural areas for a greater proportion of their lives. Contrary to other findings, pica was not significantly more frequent among sarcoidosis patients. Other elements examined by these investigators seemed not to have significance and they summed the environmental aspect of their study with the comment "One may speculate that exposure to farm animals and proximity of residences to forests may be among the more pertinent aspects of rural life. An extensive environmental questionnaire used for cooperative study by the Veterans Administration failed to reveal any important differences between sarcoidosis patients and their controls (9)

Familial studies of sarcoidosis have given rise to certain interesting findings. Since prevalence is not known Buck et al were unwilling to commit themselves strongly but their evidence as well as that of Baer Kendig et al. and Merchant and Uts suggests the definite probability that much more sarcoidosis is to be encountered among the first-degree kinship of sarcoidosis patients than might be

From the Bureau of Tuberculosis, the City of New York Department of Health, New York

Prevalence and Demographic Characteristics of Sarcoidosis in New York City

Arthur B. Robins, Hans Abeles and Aaron D. Chaves

Since 1956, the Bureau of Tuberculosis, New York City Department of Health, has been keeping records of the prevalence of sarcoidosis found in apparently healthy population groups which have had survey chest photoroentgenograms for the detection of tuberculosis. The demographic characteristics of the population studies were quite variable and probably representative of the general population. Unfortunately complete details of the age, race and sex of the surveyed population were not always available due to technical difficulties. However, using the information obtained, plus other related data such as that collected from the census of 1960, sampling techniques, and an analysis of our sarcoidosis registry, the present report was prepared in the hopes that, in spite of many shortcomings, it might prove useful in a world-wide epidemiologic review of this disease. The diagnosis of sarcoidosis was based primarily on roentgenographic examination, although confirmation of the diagnosis by biopsy or Kveim test was accomplished in over 50 per cent of the cases. On the basis of this experience, it would be fair to state that

Table I. Sarcoidosis and pulmonary TB findings during mass X-ray surveys, New York City 1956—1962

Name of X-ray survey district and year	Number of persons X-rayed	Sarcoidosis findings: Number	Sarcoidosis findings: Rate per 100,000 X-ray	New Pulm. TB findings: Number	New Pulm. TB findings: Rate per 100,000 X-rays	Estimated per cent non-white %
Bedford, 1956	81,714	57	70	137	168	80
Crown Heights, 1956	12,122	0	0	9	74	32
Sunset Park, Bay Ridge, 1957	90,777	6	7	73	80	1
East Harlem, 1958	31,935	15	29	[illegible]	96	23
Morningside, 1960	87,193	[illegible]	57	93	107	95
Riverside, 1961	27,246	9	33	21	77	18
Brooklyn Neighborhoods, 1961	39,909	12	30	24	60	
Manhattan Neighborhoods, 1962	31,835	9	28	20	63	
Bronx Neighborhoods, 1962	26,874	16	60	31	115	

On the basis of the 1960 U.S. Census.
Sum total of several small surveys with wide variations in the percentages of non-white population.

milk as the sole possible medium of dissemination of unclassified mycobacteria. But if these organisms have a relationship with sarcoidosis and if milk is a major medium of dissemination sarcoidosis might be expected in nations in which the per capita consumption of milk is high, in rural areas where milk is obtained from the family's cow in irregularly distributed and perhaps limited areas where tuberculin reactive animals without gross lesions present a high degree of prevalence.

The worldwide distribution of sarcoidosis, so far as it has been described is not out of keeping with this premise And a study begun by our group has revealed so far that 20 of 22 patients with established diagnosis of sarcoidosis consumed large quantities of unpasteurized milk during their early years in rural surroundings.

In this discussion no account has been taken of the milieu intérieur of the patient himself This unknown and subtle factor may very well be the determinant of the manifestation of disease, whatever the agent and however it may be conveyed.

References

1 Comstock, G W Keltz, H., and Senger D J Clay eating and sarcoidosis A controlled study n th state of Georgia Am. Rev Resp. Dis. 84 05 S pplement 130 1961

2 Dunner E., and Williams, J H. J Epidemiology of sarcoidosis in the United States. Amer Rev Resp. Dis. 84 05, supplement, 163 1961

3. Cummings, M. M., and Hudgins, P C. Chemical constituents of pine pollen and their possible elationship to sa rcoidosis. Amer J Med. Sci. 236: 311 1958.

4 Lindner A Kutlam. A. T Sokatch, J R., and Hammarsten J F Experimental granuloma formation w th pine pollen extract. Exper and Molec Path. 1 470 1962

5. Vogel, R. A., and Thrash, A. M. Pine pollen granuloma in animals. Amer Rev Resp. Dis. 84 05, supplement, 81 1961

6. Buck, A. A. Epidemiological investigations of sarcoidosis. I Introduction Material and Methods. Amer J H g 74 137 1961

7 Buck, A. A., and Sartwell, P E. Ibid. II Skin sensitivity and en ironmental factors. Ibid, p. 152.

8. Buck, A. A., and McKusick, V A., Ibid, III, Serum proteins Syphilis Association with tuberculosis Familial aggregation. Ibid p. 174

9 Crutcher J C., and Williams, J H., Jr V A. Cooperative study of sarcoidosis Epidemiological study of individual patients. Transactions of the 20th Research Conference in Pulmonary Disease. 278, 1961

10 Kendig, E. L.: Sarcoidosis in children. Amer Rev Resp. Dis. 84 05 supplement, p. 49 1961

11 Hammarsten, J F Personal communication.

12. Dickie, H. Personal communication.

13 Palmer C. E. and Edwards, L. B. New concepts regarding the significance of tuberculin sensitivity Proceedings of the XVIth International Tuberculosis Conference, p. 373, 1962.

14 The Arden House Conference on Tuberculosis, Public Health Service Publication, No. 784 Figure 26, p. 38.

15. Nissen-Meyer S. Epidemiological studies of t berculin sensitivity III Estimations of prevalences of mycobacterial infections from results of skin tests w th mycobacterial antigens. Am. J Hyg 72: 169 1960.

16. Feldman, W H. Avian tubercle bacilli and other mycobacteria. Their significance in the eradication of bovine tuberculosis. Amer Rev Resp. Dis. 81 666 1960.

17 Smith, D T Johnston, W W Cain, I M and Schumacher M.: Changes in the tuberculin pattern in students between 1930 and 1960. Amer Rev Resp. Dis. 83: 213, 1961

18. Shook, B., Hammarsten, J F Goldsmith, J C and Cummings, M. M. Sarcoidosis in Oklahoma Epidemiology and clinical manifestations in 60 patients. Transactions of the 21st Research Conference in Pulmonary Disease. p. 186, 1962

19 Mallman, W L., and Mallman, V H Discussion at International Conference on Mycobacterial Antigens. Airlie House Va. 1963.

20. Mallman, W L. A progress report of t berculosis research at Michigan Stat University Proceedi gs Sixty Fifth Annual Meeting of the United States Li estock Sanitary Commission. p. 174 1962

21 Chapman, J S. and Bernard, J S. Contribution to epidemiology of unclassified mycobacterial infection. (Abst.) Amer Rev Resp. Dis. 88 129 1963.

22. Scammon, L. A., Pickett M. J Froman, S. and Wall, D W Non-chromogenic acid-fast bacilli isolated from tuberculous wine bear relation to M. vium and the Battey type of unclassified mycobacteria. Amer Rev Resp. Dis. 87 97 1963

From the Bureau of Tuberculosis, the City of New York Department of Health, New York

Prevalence and Demographic Characteristics of Sarcoidosis in New York City

Arthur B. Robins, Hans Abeles and Aaron D. Chaves

Since 1956, the Bureau of Tuberculosis, New York City Department of Health, has been keeping records of the prevalence of sarcoidosis found in apparently healthy population groups which have had survey chest photoroentgenograms for the detection of tuberculosis. The demographic characteristics of the population studies were quite variable and probably representative of the general population. Unfortunately complete details of the age, race and sex of the surveyed population were not always available due to technical difficulties. However using the information obtained, plus other related data such as that collected from the census of 1960, sampling techniques, and an analysis of our sarcoidosis registry the present report was prepared in the hopes that, in spite of many shortcomings, it might prove useful in world-wide epidemiologic review of this disease. The diagnosis of sarcoidosis was based primarily on roentgenographic examination, although confirmation of the diagnosis by biopsy or Kveim test was accomplished in over 50 per cent of the cases. On the basis of this experience, it would be fair to state that

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Bedford, 1956	81 714	57	70	137	168	[illegible]
Crown Heights, 1956	12,128	0	0	9	74	32
Sunset Park, Bay Ridge, 1957	90,777	6	7	73	80	1
East Harlem, 1959	51,935	15	29	[illegible]	96	25
Morningside, 1960	87 180	[illegible]	57	93	107	85
Riverside 1961	27,346	9	33	21	77	18
Brooklyn Neighborhoods, 1961	39,909	12	30	24	60	†
Manhattan Neighborhoods, 1962	31 833	9	28	20	63	
Bronx Neighborhoods, 1962	26,874	16	60	31	115	

* On the basis of the 1960 U.S. Census.
† Sum total of several small surveys with wide variations in the percentages of non-white population.

TABLE II Sarcoidosis and Pulmonary TB Findings During Mass X Ray Surveys, New York City 1956—1962 by Percentage of Non-White Population

Estimated per cent non-white[1] population		Number of persons X-rayed	Sarcoidosis findings: Number	Sarcoidosis findings: Rate per 100,000 X-rays	New Pulm TB findings: Number	New Pulm TB findings: Rate per 100,000 X-rays
40	and over	173 735	111	64	232	79
20	to 39	102,098	33	32	88	86
0	to 19	173 772	30	17	138	134
	Total	449,605	174	39	458	102

On the basis of the 1960 U.S. Census.

in our X ray surveys the *roentgenographic diagnosis of sarcoidosis* may be considered to be correct in over 90 per cent of the cases.

The present report summarizes our experiences from 1956 through 1962.

Findings

No definite yearly or seasonal variation in the prevalence of sarcoidosis was evident from our studies. There were striking differences, however according to race or ethnic groups. This can be best shown by combining all the surveys, and analyzing the results in accordance with the racial composition of the population.

In Table I the results of mass surveys from 1956 to 1962 inclusive are tabulated comparing sarcoidosis rates with the rates of newly found cases of active pulmonary tuberculosis. It should be realized that the estimated percentage of non-white population is based on the 1960 U S census in which 95 of the Puerto Rican population was allocated to the white group and 5 to the non-white (Negro) The findings are tabulated in the order surveys were carried out since 1956

In Table *II* the same reports are analyzed according to the percentage of non-white population in the survey area. The results are divided into three groups, the percentage of non-white population being 0—19 20—39 and 40 % and over in the respective groups. The findings in all three groups are based on

TABLE III Sarcoid Cases Registered from the Chest Clinics of the Bureau of Tuberculosis By Age, Sex and Ethnic Distribution

Sex and Age Group	Negro	Puerto Rican	White	Total
Male Total				
Under 19	4	0	2	6
20—29	*49*		2	53
30—39	29	5	6	40
40—49	6	0	6	12
50 and over	7	2	3	12
	95	9	19	123
Female Total				
Under 19		2	0	7
20—29	44	2	4	50
30—39	37	9	12	58
40—49	19	6	8	33
50 and over	14	2	4	24
	119	21	28	168
Sex Combined Total				
Under 19	9	2	2	13
20—29	93	4	6	103
30—39	68	14	18	98
40—49	25	6	14	45
50 and over	21	4	7	32
	214	30	47	291

more than 100,000 persons in each group. There is an increase in the sarcoidosis rate per 100,000 X-rays from 17 in the group with the lowest percentage to 64 in the one with the highest percentage of non-white population.

During the past four years the New York City Department of Health has kept register of all sarcoidosis cases examined in its chest clinics. This includes survey and non-survey cases (Table III). Of the 291 registered cases, 73.5 are Negroes, 16.2 white and 10.3 of Puerto Rican origin. The marked preponderance of Negro cases agrees with the trend brought out by the survey findings in Table II. 57.7% of the 291 sarcoidosis cases are females and 42.3% males. The relatively large proportion of males in this group is probably due to the fact that (1) many cases of the register were reported from tuberculosis mass surveys which are directed toward the older male population and (2) from army induction examinations. The age distribution shows the largest numbers of cases in the 20—29 and 30—39 year groups.

Since the base population from which the registered sarcoidosis cases originated is not known, no conclusions can be drawn from these figures, but they may indicate trends which may be of assistance for more detailed studies.

From the Hospital de Clínicas Dr Manuel Quintela Montevideo, R. O del Uruguay

Epidemiology of Sarcoidosis in South America

Pablo Purriel, Eduardo Navarrete and Aristeo Piaggio

In order to obtain epidemiological data on sarcoidosis in South-America, we have employed two methods

1 To send the questionnaire of Drs. Bauer and Löfgren to leading chest specialists in the different South American Countries.

2 To review the available literature on the subject and thus become acquainted with cases reported in different countries.

It is our impression that although the problem of sarcoidosis in South-America is not of the same importance as in certain areas of the U.S.A or Sweden, the data currently available are not conclusive as to the real significance of the problem.

The following is a survey of results

Argentine

Department of Tuberculosis. Survey of University Students

Prof. J C. Rey University of Buenos Aires. Examinations on admittance and periodical controls.

Center for tuberculosis control

Rosario — Argentine.
Dr Schottlender

Number of persons examined 130 000
Number of sarcoidosis cases 0

Cordoba

Prof José A. Pérez.

Mass screening data include no information on sarcoidosis. Only isolated clinical cases are known.

Age From 17 to 25 years. White race

Periods	Examination	Number of students	Sarcoidosis Number	Sarcoidosis per 100,000
1941—52	At enrolment	82,800	3	3,62
	Re-examination	31,500	3	9,52
1953—57	At enrolment	90 000	5	5,55
	Re-examination	19 400	0	0.0
1958—60	At enrolment	40,000	2	4.0
	Re-examination	23,000	4	17.39
1961—63	At enrolment	40 000	0	0.0
	Re-examination	13 000	0	0 0

Compatible with sarcoidosis 12 Sarcoidosis (with biopsy) 3

Available literature from Argentine comprises 60 cases reported by various authors.

Conclusion. Mass survey fails to provide composite picture of the sarcoidosis problem in Argentine.

However the existence of such problem is clearly indicated by the data collected and reported on by Dr Rodriguez Castells.

Argentine league for TB control

Dr Rodriguez Castells. Buenos Aires.

Year	Number of persons examined	Cases of sarcoidosis
1954	76,639	1
1955	81 425	2
1956	81,784	1
1957	92,312	1
1958	84,991	1
1959	64,576	—
1960	67,575	—
1961	84,995	1
1962	61,019	—
	Total 695,312	7

Dr Rodriguez Castells made an inquiry in Argentina among 699 chest physicians. Replies were received from 89 of these, and 100 cases of sarcoidosis were reported.

Brazil

Population 66,000,000.
Negroes 10 %
Mulattos 26 %

100 cases of sarcoidosis studied in Argentina

Collected by Dr Rodriguez Castells.

Nationality		Sex		Age		Number	Positive biopsy	Without biopsy
Argentinians	82	Male	61	Under	20	7	84	16
Spanish	8	Female	39	From	20 to 29	16		
Italian	8			From	30 to 40	39		
Yugoslavian	1			Over	40	38		
German	1							

Sao Paulo. Hospital das Clínicas. Dr D. A. Certain. 1961—62. 111,870 patients admitted. Sarcoidosis 0.

Service for Tuberculosis Control. Serviço de Tuberculosis. Prefeitura Municipal. 1955—63. 52,861 examinations. Sarcoidosis 1 case.

Research Institute "Clemente Ferreira" 1960—63. 210,000 examinations. Sarcoidosis 2 cases.

Sesi. Serviço Social da Industria. 1947—63. 1,500,000 examinations among industrial workers. Sarcoidosis 1 case.

Data furnished by Dr S. Bemol de Amaral. Heart Institute. 47 450 patients admitted. Sarcoidosis 0.

Rio de Janeiro

Prof. Aloysio de Paula

Sarcoidosis diagnosis is performed in a few centers. Knowledge of the disease is not a part of medical practice.

Dr José Machado Filho:

No epidemiological investigation available. Only isolated clinical cases are known.

Bahia

Prof. J. Silveira. No information on Sarcoidosis was obtained from MR surveys.

Manaus — Amazonas

Amazonas National Research Institute.
Director Dr Djalma Batista.

Mass Survey carried out in Manaus disclosed no cases. However Dr Batista is aware of 1 case (Symptomatic)

SOUTH AMERICA

POPULATION 123.000.000

EPIDEMIOLOGY OF SARCOIDOSIS

PURRIEL NAVARRETE PIAGGIO

TB CASES

80 CASES

CENTER FOR TUBERCULOSIS CONTROL

NUMBER OF PERSONS EXAMINED	NUMBER OF SARCOIDOSIS CASES
130 000	0

ARGENTINE LEAGUE FOR TB CONTROL

YEAR	NUMBER OF EXAMINED	CASES OF SARCOIDOSIS
[illegible]	76 639	1
[illegible]	81 423	2
[illegible]	81 784	1
[illegible]	82 312	1
[illegible]	84 997	1
[illegible]	84 576	
[illegible]	67 573	
[illegible]	84 395	1
[illegible]	51 019	
TOTAL	694 312	7

August 1963

DEPARTMENT OF TUBERCULOSIS SURVEY STUDENTS

PERIOD	EXAMS	NUMBER OF STUDENTS	SARCOIDOSIS	
			NUMBER	PER 100000
1944 48	AT ENROLMENT	82 800	3	3,62
	RE EXAMINATION	31 500	3	9,52
1953 57	AT ENROLMENT	90 000	5	5,56
	RE EXAM	19 400	0	0,0
1958 60	AT ENROLMENT	40 000	2	5,0
	RE EXAM	23 000	4	17,39
1961 63	AT ENROLMENT	40 000	0	0,0
	RE EXAM	13 000	0	0,0

100 CASES OF SARCOIDOSIS STUDIED IN ARGENTINE

NATIONALITY	SEX	AGE	NUMBER	[illegible]	[illegible]
ARGENTINIAN 82	MALE 61	UNDER 20	7	84	16
SPANISH 8	FEMALE 39	FROM 20 to 29	16		
ITALIAN 8		FROM 30 to 40	39		
[illegible] 1		OVER 40	38		
[illegible] 1					

Available literature from Brazil comprises 18 cases. Recent congresses hav dealt with the disease.

Chile

W hav not been able to obtain data from mass surveys. It is our impression that sar coidosis is little known here. Only 2 cases have been reported in the literature. Chile has extensive pine woods covering over 400,000 acres.

Bolivia

La Paz. — Dr Santiago Mederos. Only 1 case of sarcoidosis is on record.

Peru

Lima. Dr García Rosell. — No epidemiological survey on sarcoidosis has been carried out.

Equado

Guayaquil. — Dr Jorge A. Higgins. — No cases of sarcoidosis known to date.

Colombia

Bogotá. — Prof. C. Arboleda Díaz. — No epidemiological investigations on sarcoidosis.

Venezuela

Maracaibo. — Dr Pedro Iturbe. — No epidemiological survey on sarcoidosis has been carried out.

References

Purriel, P. Navarrete, E. Epidemiology of Sarcoidosis in Uruguay and other countries of Latin-America. Am. Rev. Resp. Dis. 84 (2) 155—61 1960.

Purriel, P. Navarrete, E. Epidemiologia de la Sarcoidosis en el Uruguay. El Tórax, IX, 194—199, 1960.

Purriel, P. Navarrete, E. Datos epidemiológicos sobre sarcoidosis en América Latina. El Tórax, IX, 200—203, 1960.

(For other references regarding sarcoidosis in South-America see bibliographies of the papers listed above.)

From Departamento de Doenças do Torax da Policlínica Geral do Rio de Janeiro, Brasil.

Sarcoidosis in Brasil

Aloysio de Paula

The first case of sarcoidosis was published in Brasil in 1939 by Prof. F. E. Rabello. Although the symptoms of this disease are well known by clinicians and specialists, the amount of cases published so far are still only a few. Nevertheless, everything leads us to believe that sarcoidosis is just as frequent in Brasil as in the United States. In this paper — which is a summary of a report prepared for the VII Brasilian Congress of Chest Diseases — the author selected 25 cases, in which biopsies were performed, with the exception of only one case.

The author agrees with Löfgren's concept, which sets apart completely the sarcoid reactions found in a series of diseases — tuberculosis, leprosy, mycosis, beryllosis, cancer, etc. — from sarcoidosis itself, a disease which is known to have its own characteristics, and which has a still unknown etiology.

The diagnosis of sarcoid lesions is possible only through a combination of elements, like the clinical picture, its evolution, pulmonary radiological picture, pathological anatomy, tuberculin reaction, laboratory tests and response to corticotherapy. The diagnosis is finally reached at by the combination of these elements and no value should be given to one finding alone.

In practice we should pay special attention to the clinical picture, the radiological picture, the pathological anatomy and the tuberculin test. The tuberculin test assumes a great importance in Brasil, due to the high TB prevalence still existent. If it becomes impossible to put in evidence the classic sarcoid granuloma, the other elements should be evaluated, and greater emphasis should be placed upon the clinical evolution.

Sarcoidosis in Brasil is diagnosed only in the bigger cities, where a larger number of specialists is found. Its identification calls for a greater amount of medical knowledge as well as the use of special tests and means, seldom found in smaller cities. The disease will only be recognized all over Brasil through a large educating campaign specially designed to call attention of clinicians and specialists to its peculiar aspects. At this moment, the biggest difficulty for the diagnosis lies in its spontaneously curable forms, sometimes unnoticed. Sometimes group radiological examination may lead to chance detection. In well identified cases, it is often suggestive of other diseases by the consequences of its advanced stages, like pulmonary fibrosis and emphysema, pulmonary and cardiac insufficiency. In Brasil, several published cases were recognized by autopsies or by exploratory surgery performed to detect obscure or unidentified diseases.

COMMENT

Dr Viragh: Dr Carneiro from Porto Alegre in the state of Rio Grande do Sul, Brasil, observed 21 patients with sarcoidosis in the last five years. He sent the tabulation of his cases to me for reporting to this conference. Brasil is the only other country with proportion of Caucasians and Negroes comparable to that in the United States where the prevalence of sarcoidosis among Negroes is an established fact. This prevalence was found in Carneiro material also.

From the Hospital de Clínicas Dr Manuel Quintela Montevideo, R.O del Uruguay

Epidemiology of Sarcoidosis in Uruguay

PABLO PURRIEL, EDUARDO NAVARRETE and ARISTEO PIAGGIO

Uruguay is situated on the Southern coast of the American continent, between parallels 30 and 35. It has an area of 187 000 sq kilometers, with population of 2,500,000.

Discrimination between urban population and rural inhabitants is rather difficult. As many as 1 million persons live in the capital, Montevideo. The population is made up of the white race, with only 10,000 negroes.

Uruguay was one of the first countries in South-America to start mass survey programs with mobile units (1948) BCG vaccination has been practiced since 1927 Up to 1963 1 492,202 persons were accinated, 563,734 from urban and 928,448 from rural areas.

From 15 to 20 % of the population attend annually the different screening centers located throughout the country

Below is an example of mass survey conducted in conjunction with examination for sarcoidosis and tuberculosis.

Other examples might have been given which show similar results. Hence it is concluded tha under prevalent mass survey procedures, thorough assessment of sarcoidosis incidence is not feasible.

In our own Mass Survey MR films are interpreted by group of practitioners called readers" who merely prepare simple radiological report, which is sent to the examinee private physician.

However as the understanding of sarcoidosis is not, as yet, part of routine medical practice, only a few physicians are acquainted with the disease. X-ray reports on mass surveys, involving the presence of sarcoidosis, are related to other etiologies, mainly tuberculosis.

I our sarcoidosis material 22 cases were diagnosed in mass survey as tuberculous lesions. Subsequently the true etiology became known sarcoidosis.

W have carried out thorough investigation throughout the country in order to ascertain the sarcoidosis prevalence. This has resulted in our gaining the impression that numerous cases of sarcoidosis have not been diagnosed and are treated for tuberculosis.

	Mass X-ray Survey	*Uruguay*	*Urban areas*
Date From — to	Satisfactory 70 mm films	Suspected actv TBC	Sarcoidosis No. per 100,000
Jul./52—Oct./57	1,077,180	318.3	— —
	Mass X-ray Survey	*Uruguay C.L.H.A.*	*Rural areas*
Date From — to	Satisfactory 70 mm films	Suspected actv TBC per per 100,000	Sarcoidosis No. per 100,000
Apr./51—Nov 62	1,838,915	364.9	8 0.4

From Departamento de Doenças do Torax da Policlinica Geral do Rio de Janeiro, Brasil

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	Mass X-ray Survey	*Uruguay*	*Urban areas*	
Date	Satisfactory	Suspected	Sarcoidosis	
From — to	70 mm films	active TBC	No. per 100,000	
Jul /52—Oct./57	*1,077 180*	548.5	—	—
	Mass X-ray Survey	*Uruguay C.L.H.A.*	*Rural areas*	
Date	Satisfactory	Suspected	Sarcoidosis	
From to	70 mm films	active TBC per per 100,000	No. per 100,000	
Apr /51—Nov 62	1,838,915	564.9	8	0.4

Up to August 1963 90 cases of sarcoidosis had been studied by us. Of these, 12 proved fatal, 26 were cured and 40 are still under treatment. We do not know the results of the follow-up investigations of 12 patients. Sixty cases represent the current prevalence of sarcoidosis in Uruguay the rate being 2 4 per 100 000

Race

91 1 %	white
8.9 %	negroes
100 0 %	total

If we take into account the low percentage of negroes among the Uruguayan population (0 4) the importance of the race factor becomes apparent.

Sex

51.5	females
48.9 %	males
100 0 %	total

These figures are rather significant, since most statistics show a distinct prevalence of females.

Origin

57 8 %	urban areas
42 7 %	rural areas
100 0 %	*total*

Contrary to data from other countries such as USA and Sweden, where rural predominance is definite, our indices show a slight urban prevalence. However it should be noted that in this country the urban population is larger than the rural

Incidence

Incidence in the group w studied was as follows

1949—1951	13 cases
1952—1954	7
1955—1957	22
1958—1960	21
1961—1962—1963	27
Total	90 cases.

The increase in the number of cases recorded during the last few years is undoubtedly related to a better knowledge of the disease on the part of the practitioners.

Age

From 14 to 19 years	5 cases		5.6
20 » 29	23		25.6
30 » 39 »	14		15.6 %
40 » 49 »	21		23.3 %
50 » 59 »	20		22.2
60 » 69 »	5	»	5.6
over 69 »	1	»	1 1 %
unknown »	1		1 1
Total	*90*		*100 0*

Diagnosis

Eighty two biopsies were performed, with the following results

Biopsy

		Rate %
Positive	71 cases	86.6
Negative	11	13.4
Total	*82*	*100*
Without biopsy	8	8.8
With biopsy	82	91.–
Total	90 cases	100 0

Radiologic classification (According to first examination)

	Cases	Percentages
Hilar adenopathy	32	35.6
Hilar adenopathy plus nodules	12	13 3
Hilar adenopathy plus miliary lesions	5	5.6
Disseminated miliary lesions	4	4 4
Diffuse pulmonary nodulation	14	15.6
Fibrosis	4	4 4
Normal	19	21 1
	90	*100.0*

Clinical symptomatology during the course of disease.

Asymptomatic cases	15—16.6 %	
Symptomatic cases	75—83.3 %	
Total	90	100.0 %

According to localization.

	No. of cases	Percentage
Thoracic	18	20
Extrathoracic	17	18.9 %
Mixed	55	61.1 %
	90 cases	100 %

In Uruguay along the River Plate coast, there are pine forests with total area of about 35,000 acres, covering a 150 km. belt. Planting started over 60 years ago 90 % of the trees belong to the "pinus maritimus" or "pinus pinaster" varieties.

Only 6 patients were from this area, thus disproving the connection between pine-forest environment and sarcoidosis in Uruguay

W have just completed an epidemiologic investigation among the general population, involving 30,000 intradermal reactions with histoplasmine supplied by Lilly Laboratories 10 to 15 ga positiv reactions.

Biologic reactions.

Comparison of tuberculin allergy investigated in mass surveys, with that found in sarcoidosis cases.

	Total	Positive allergy	Negati allergy
General population (from 10 to 69 years)	444,078	350,286	93,792
Frequency	100	78.8 %	21.2 %
Sarcoidosis (from 14 to 69 years)	83	13	70
Frequency	100	15.7	84.3 %

Of the 83 cases investigated, 41 gave negative Pirquet
27 negative Mantoux,
2 negative BCG,
13 positive Pirquet

83

Pine pollen sensitivity in sarcoidosis

Number of sarcoidosis cases investigated	Dilution of pollen 1/1000	1/100	Negative	Positiv [illegible]
15	7	8	5	0

Histoplasmine
(Intradermal reactions)

No. of sarcoidosis cases investigated	Positive
60	0

TABLE I Kveim's test in active and inactive sarcoidosis

Kveim	Positive		Negative		Total	Rate per 100	
						Positive	Negative
Biopsy	Pos.	Neg	Pos.	Neg			
Active	12	1	4		17	76.5	23.5
Inactive	4		14	3	21	19 0	8.0
Total	16	1	18	3	38		

Kveim's test

This test was performed on 38 patients 32 of them showed positive histological tests and 6 negative, or were not biopsied.

With regard to the grade of activity of this disease, it should be stated that 76.5 of Kveim's tests proved to be positive whereas only 19 of the non-active forms were positive as shown in Tab. I

Of all the countries of the Western Hemisphere, Uruguay has the largest number of sarcoidosis cases in proportion to its population. Nevertheless, we believe that a considerable number of cases of this disease remain undetected

A better knowledge of this disease, particularly among physicians engaged in the interpretation of MR films would be most useful.

From the University Hospital Cairo, Egypt (U.A.R.)

Sarcoidosis in Egypt (U.A.R.)

TAHA GOMAA

Though Mass Radiography has been introduced into Egypt since 1949 no special study has been made so far to find out the incidence or prevalence of sarcoidosis in Egypt. It seems that either the disease is not very common, and consequently does not attract the attention of the clinicians or radiologists, or what is more likely the disease entity of sarcoidosis is not well known by these workers. Hilar adenopathy is always diagnosed primarily as of tuberculous origin; and whenever confirmation is lacking (e.g. tuberculin anergy) it is then the reticulosis group of diseases which most frequently come to question. It is only by chance that sarcoidosis is thought of, especially when the biopsy report confirms the diagnosis.

It is only very recently that an attempt has been made by Saad and co-workers, 1962, to collect data about the prevalence of sarcoidosis. This was done by sending questionnaire to physicians engaged in various specialized fields (chest specialists, general practitioners, radiologists, pathologists, dermatologists and ophthalmologists etc.) to inquire about their experience as regards the frequency of the disease in their own practice. Although this is far from giving proper idea of the prevalence or incidence of the disease in Egypt, it has led, however to 25 cases being reported. Of these 10 were confirmed histologically 13 were very suggestive and two were doubtful.

In this report another attempt is made to obtain further information on the disease. The material used was collected from the University Hospitals (capacity 3000 beds) of Cairo University.

During the last 10 years (July 1952—June 1962) 29 cases of sarcoidosis were recorded, all of which were histologically confirmed.

Case Analysis

Sex. Out of 29 patients, 20 were males, and 9 females. This preponderance of males is artificial, as the number of beds for male patients in the hospital is twice that for female patients. If we take this point into consideration, sex distribution will be almost equal.

Age: It is interesting to note that the youngest patient was 8 months old, and the symptoms exhibited were in the form of skin lesions. The oldest patient was 60 years of age.

Though the number of cases is very small, it is nevertheless noticeable that patients were mostly affected between the ages of 30—50 years.

Clinical Features

The symptoms are manifested in three main ways.

(a) Pulmonary Symptoms

Cough and dyspnoea are the main features of this group. Sputum is either scanty or absent. The dyspnoea is especially evident on the slightest exertion. This group comprised 8 cases.

b) Lymph Node Enlargement

This group is always mistaken for some form of lymphoma, or blood disease. Diagnosis can be established only by biopsy and histological examination. There were 9 cases that belonged to this group.

(c) Skin Lesions

Skin lesions were the most frequent symptoms in the whole series, and were observed in 12 cases. These skin lesions were distributed either over the whole body or affected only certain parts, particularly the face, e.g. forehead, nose, chin and cheeks. They were fre

Table I Organs that showed signs of involvement

Organ involved	No. of cases
Lungs	12
Hilar glands	14
Superficial glands	11
Skin	13
Spleen	4
Parotid gland	1
Others	1

quently mistaken for Leishmaniosis, or Lupus vulgaris.

One case was interesting in its mode of manifestation acute intestinal obstruction. It was diagnosed only after operation, and was confirmed histologically

Site of Lesions

Table I shows, as far as the clinical picture allowed, the organs that were mostly involved. No liver biopsy was performed in any of these cases. A radiological picture of the osseous system was made only in a few cases.

Five of the cases that showed generalized lymph node enlargement were associated with hilar adenopathy and 4 of the dermatological cases with pulmonary or hilar gland involvement.

Kveim Test

All cases were confirmed histologically by biopsy taken from either a superficial gland prescalene node, or skin lesions. In 4 cases additional confirmation was obtained by a Kveim test.

Racial and Climatic Factors

No particular racial or climatic factors could be observed. No pine trees exist in Egypt, and all the patients were of the same race

Discussion

Neither the number nor the type of cases presented in this series can represent in any way the epidemiological condition of sarcoidosis in Egypt. Sarcoidosis is not a notifiable disease, nor are its diagnosis and clinical features well recognised by the majority of physicians in the country However this report is an attempt to throw a certain amount of light on all that is known about the disease in Egypt. It is hoped, that in the near future a more detailed report will be made. At present a pilot-project for tuberculous control is being conducted in a semiurban and rural area near Cairo. The total population of this area is about 250 000 All the inhabitants are examined photofluorographically and tuberculin tests are administered. This survey will enable us to pick out all the cases, where hilar adenopathy is combined with anergy to tuberculin, for further investigation and follow up studies on sarcoid disease.

However as far as the present series is concerned, the following conclusions can be drawn

1 The disease "Sarcoidosis" does occur in Egypt.

2 So far it is not a common condition, compared with other diseases, e.g tuberculosis.

3 Its manifestations are mainly in the skin, hilar glands, pulmonary parenchyma and superficial lymph nodes.

4 Biopsy should be made in all cases that exhibit all or any of the above-mentioned features.

5. The desirability should be stressed of the disease being more readily recognized by particularly chest specialists, dermatologists, and general practitioners.

6. A more serious attempt should be made to ascertain the exact degree of prevalence and the incidence rates in the general population.

References

1 Samid, A., Abdel Hakim, M and Salem, E. J Egypt. Med. Assoc. Vol 46, No 2 1963.
2. Rabert, A. S Post graduate Medicine Vol. 3, p. 232 1962.
3. American Review of Resp. Diseases. (special number) Vol. 84 No. 5. Part II

From the Pulmonary Unit, Rothschild Hadassah University Hospital, Jerusalem, Israel

Epidemiology of Sarcoidosis in Israel

JOSEPH RAKOWER

In the seven-year period from 1956 to 1962, 70 cases of sarcoidosis, *histologically confirmed*, were found in Israel. The mean annual incidence was 0.5 per 100,000 (Table I). The prevalence obtained from mass X-ray examination was 1.6 per 100,000. It can be deduced from these data that sarcoidosis is rar in Israel, its incidence being 11 times lower than in Denmark (1).

As regards the reliability of the material presented, it should be noted that Israel is a small country with population of 2 1/4 millions, with highly developed medical organisation (the physician-population ratio of 1 400 is the highest in the world) and the medical facilities are easily accessible to all strata of the population in town and country.

The peak incidence occurred in the 20 to 39 year age groups. The sex distribution was 3 males to 1 female, the ratio being just the *reverse of what is found in* Europe and the U.S.A.

TABLE I. Annual incidence of sarcoidosis Israel 1956—62

Year	Population	Number of cases	Rate per million
1956	1,870,000	7	3.7
1957	1,970,000	8	4.0
1958	2,060,000	9	4.4
1959	2,090,000	12	6.0
1960	2,150,000	13	6.0
1961	2,230,000	11	5.0
1962	2,3[illegible]0,000	10	4.4

The incidence of sarcoidosis was not uniform *in various regions of Israel. In Jerusalem* its incidence rate was five times higher than that of Tel Aviv and almost four times higher than that of Haifa (Table II). Jerusalem is adjacent to the greatest concentration of pine woods anywhere in Israel. However in Upper Galilee, also forested region, the incidence of sarcoidosis was lower than in the Tel Aviv area, which has much fewer pines. The awareness of sarcoidosis, especially among chest physicians, is high in all regions of the country.

Another epidemiological problem is the prevalence of sarcoidosis among the different ethnic groups in Israel. The mass immigration to Israel from many countries in the world provides an opportunity to investigate this interesting epidemiological problem.

Differences in morbidity and mortality between European and Afro-Asian *immigrants* have been established beyond any doubt. The fact that the main causes of death in the Occident are arteriosclerosis and cancer and in *the Orient infections* and malnutrition, is well-known. It is shown in Table III that the incidence rates of infectious diseases, such as gastroenteritis or pneumonia, were 3 to 5 times higher in the Oriental group, while those of malignant neoplasms and myocardial *infarction* were 2.5 to 3 times higher in the European group. However there was no difference between the incidence rates of sarcoidosis in the two groups. In Table III the ratio between the sarcoidosis incidence rates in the Occidental and Oriental groups is far from the ratio of infectious diseases, and far

2—41324

TABLE II Incidence of sarcoidosis and coniferous forests in different regions of Israel — 1956—1962

Region	Mean population	No. of cases reported	Annual incidence rate (per million)	Coniferous forests per centage of total area
1 North	340 000	2	0.8	3.5
2 Haifa	320 000	11	5 0	2 7
3 Center	410 000	17	6.0	1.8
4 Tel-Aviv	640 000	16	3.6	0.4
5 Jerusalem	180,000	22	17 4	14.0
6 South	110 000	2	2.6	0.4
All Israel	,000 000	70	5	

TABLE III Proportion between Rates of Incidence of Selected Diseases for Afro-Asian and European Groups in Israel

(1 = the Afro-Asian Rate)

No.	Diagnosis	Age	Proportion of Rates
1	Gastroenteritis (2)	0—2	1:0.2
2	Pneumonia (2)	0—2	1·0.3
3	Asthma (3)	All ages	1·0.7
4	Sarcoidosis	20 +	1 1
5	Malignant Neoplasms (2)	65 +	1.2.6
6	Myocardial Infarction (2)	65 +	1 3 1

from myocardial infarction, t is proximal to the ratio of bronchial asthma. These data seem to be consistent w th the hypothesis that sarcoidosis is a hypersensitivity disease

W th regard to the clinical course of sarcoidosis, in the 70 patients studied the chronic persistent form was found only in 11 patients (16 %) 3 of these (4.3) died from sarcoidosis, 6 patients (8 6 %) were severely incapacitated, and 2 patients (3) were not markedly affected The causes of death were pulmonary fibrosis with cor pulmonale in 2 patients and cardiac sarcoidosis in the third patient. The causes of incapacity were pulmonary fibrosis in 5 patients and hypercalcemia in the sixth. In the latter case, the patient suffered from diffuse calcinosis, cholelithiasis, nephrolithiasis, polyarteritis calcificans, and hypersplenism w th crises of haemolytic anemia.

References

1 Horwitz, O Geographic Epidemiology of Sarcoidosis in Denmark 1954—1957 Amer Rev Resp. Dis. 89 133 1961
Blondheim, S. H and Kallner G Incidence of Diseases among the Communities of Israel. Harefuah 51 4 1956.
3 Groen, J J and Liber E. Socio-Medical Aspects of Bronchial Asthma in Israel. Isr Med. J 19 21 1960.

From the Institute of Public Health, Tokyo, Japan

Epidemiologic Features of Sarcoidosis in Japan

K. Nobechi

In addition to comment on the data contributed by Japan to the joint world-wide survey representing the Sarcoidosis Research Committee in Japan, I would like to report briefly on few items of the epidemiologic findings obtained, based on 282 cases chosen out of 450 records of sarcoidosis patients. These data were collected through the renewed nation-wide survey conducted since the former one, on which I had reported at the II International Conference in Washington in 1960.

1 The Prevalence Rate of Sarcoidosis in Japan In the joint world-wide survey the data were derived from the annual mass examinations of employees of the Japan National Railways in the Tokyo District. Taking the various factors concerned into account, these data were judged to be the most reliable and most representative of Japan, and, consequently we sent these in reply to Dr Löfgren inquiries. In the light of the standardized world-wide survey attempted by Dr Löfgren, we may now safely assume that sarcoidosis prevalence in Japan is nearly one tenth of the prevalence rate in Sweden.

2. Sex and Age Distributions were estimated by analyzing the records of the above-mentioned 282 cases. The results obtained are shown in Table I. The distribution of the two sexes was almost equal. About half of the cases were in the age group 20—29 years, and formed markedly sharp peak. However as I pointed out at the Washington Conference, the latter feature is social rather than biological phenomenon, due to the age distribution of the subjects of the mass physical examination, whereby the highest number of sarcoidosis cases were detected.

Table I Sex and age distribution of sarcoidosis cases at the time of diagnosis

Group[1]	I—III Groups		IV Groups	
Total	185 Cases	100.0 %	97 Cases	100.0 %
Male	99	53.5	46	47 4
Female	86	46.5	51	52.6
Age				
0—9	5	2.7	0	0
10—19	26	14.0	20	20.6
20—29	83	44.9	49	50.5
30—39	28	15.1	17	7.5
40—49	21	11.3	5	5.2
50—59	14	7.6	3	3.1
60—	4	2.2	1	1 1
Unknown	4	2.2	2	2.1

The examined samples, chosen out of 454 cases, and collected through the nation-wide survey by the Sarcoidosis Research Committee in Japan.

The groups according to the classification by the Medical Group at the II International Conference on Sarcoidosis in Washington, 1960.

Members of the Sarcoidosis Research Committee Kitamura (Chairman), Oka (Former Chairman) Okazaki, Donomae, Fukushiro, Higuchi, Homma, Iwasaki, Kawatomi, Kurosawa, Yabuchi, Ohnishi, Seda, Shigematsu, Shakano and Takahashi.

TABLE II. Geographic distribution of sarcoidosis cases[1] by residence at the time of diagnosis

District	Cases	Population	Rate/ 100 000
Hokkaido	13	5,039,206	0.26
Tohoku	40	9,325 699	0.43
Kanto	123	23 002,983	0.53
Central	29	16,563,243	0.18
Kinki	36	15,515 634	0.23
Chugoku	11	6,944 725	0.16
Shikoku	6	4 121 423	0.15
Kyushyu	13	12,903,515	0 10
Unknown	11	73	—
Total	282	93 418,501	0.30

As explained in table I

Living on the border of two districts.

3 Geographical Distribution. Fig 1 in Dr Hosoda's report on the pine pollen problem (page 59) represents a geographically plotted illustration of our 282 cases, showing their place of residence. The distribution of sarcoidosis is to a large extent parallel to the densities of the local populations. For instance, the extreme congestion of cases in the Eastern part of the Tokyo area results from the fact that one tenth of the whole population of Japan resides there.

However from Table II it could be inferred that the geographical distribution of sarcoidosis *might tend to be denser in the northern provinces* than in the southern. There are also some indications that the incidence of sarcoidosis may be higher for Tohoku and Hokkaido Provinces. This is what the epidemiologic study group of the Sarcoidosis Research Committee is striving to ascertain.

4 Influence of Environmental Factors. Despite our own efforts and the cooperation of health centers throughout the country no positive correlation of various environmental factors with sarcoidosis has so far been detected.

5. Erythema Nodosum. At the 1960 Conference, I reported that there was no case of erythema nodosum among 94 cases. Up to date we observed only one case in our material. Thus, the rarity of erythema nodosum is still true here as in America.

From the Central Chest Clinic, Melbourne, Australia

Prevalence of Pulmonary Sarcoidosis in the State of Victoria, Australia

R. S. A. Marshman

The figures presented relate to the State of Victoria, Australia, and are derived from annual returns of mass chest X-ray surveys carried out throughout the State for the four years from 1959 to 1962. Diagnosis was by radiological evidence only.

Mass Chest X-ray Surveys have been carried out in this State on a voluntary basis for all people aged thirteen years and over since 1948, and for the past ten or twelve years approximately 400,000 people have been X-rayed annually. Visits of the Mass X-ray Division are made to Melbourne approximately each two years whereas in the country they vary but would average each three years. It is estimated that at least 55 % of the population has been X-rayed within the previous years. The figure may be higher.

The State of Victoria is situated in South-Eastern Australia. *The climate is temperate.* Compared with many other parts of the

Table I. Prevalence of pulmonary sarcoidosis in mass chest radiography in the state of Victoria, Australia

Year	Persons X-rayed	Persons sarcoid	Rate per 100,000
1959			
Total	401,568	45	11.2
Male	210,694	13	6.1
Female	190,874	32	16.7
1960			
Total	380,598	38	9.8
Male	198,532	18	9.0
Female	181,066	20	11.1
1961			
Total	392,052	27	6.9
Male	217,542	22	10.1
Female	174,510	5	2.8
1962			
Total	396,773	35	8.8
Male	217,037	13	5.9
Female	179,736	22	12.2

Table II. Age distribution of pulmonary sarcoidosis detected by mass chest radiography in the state of Victoria 1959–1962

Age years	Males	Females	Total
15—19	5	10	15
20—24	10	9	19
25—29	10	6	16
30—34	12	17	29
35—39	6	14	20
40—44	3	8	11
45—49	4	6	10
50—54	4	5	9
55—59	4	1	5
60—64	2	2	4
65—69	3	1	4
≥ 70	2	—	2
Unknown	1	—	1
	66	79	145

world it is sparsely populated. The population at present is approximately three million people, two-thirds of whom are living in the capital city Melbourne. The people are almost entirely of European extract and at June 1961 Census 81 % of Victoria's population were born in Australia. Of the remainder 12 % were British born outside Australia, and 7 % were born elsewhere. Most of the State is given to rural pursuits, but a proportion is still under forest, chiefly eucalypt. There is a species of native pine which grows in Northern Victoria, but from the earliest days of colonization pinus radiata was introduced with holdings as they were developed, chiefly for ornamental or wind-break purposes, and so specimens of these trees are scattered through the greater part of developed areas in the State. In addition to this in more recent years (30 or 40 years) softwood forests have been developed in isolated areas and these consist chiefly of pinus radiata.

During the four year period, 1959—1962, *1,571 011* chest X-rays were recorded, *145* cases of sarcoidosis were diagnosed, which gives a rate of 9.2 per 100,000. The incidence was higher in people aged between 15 years and 40 years, but there was no definite sex relationship. Records from other Australian States are fairly comparable.

From the Department of Pathology Wellington Hospital, New Zealand

M.M.R. Survey of Sarcoidosis in New Zealand

J D Reid

The data presented here have been kindly provided by Dr C. H. King, Auckland, Dr M. C. Laing, Wellington, and Dr F de Hamel, Christchurch, and represent figures from the three largest Mass Miniature Radiography (M.M.R.) Units in the country

The differences in data from these areas are so great that the reports are presented separately (Table I) A great many factors might be responsible for the disparities shown. Whereas in Auckland general population survey was reported, selected groups have always been covered in Wellington, and also in the past few years of the Christchurch work. The two northern units gave urban coverage whereas that in Christchurch was mixed. Diagnosis in Auckland was in the majority of cases on radiological basis only while in Wellington and Christchurch almost all cases were investigated in Hospital and majority had biopsies For Wellington certain amount of Kveim test material was also available.

The average attendance of those invited to be examined was 70 in Wellington and this could probably be accepted for other centres also The rate of re-examination was found in Christchurch in 1960 to be 70 i.e. only 30 were being examined for the first time. In Wellington the figure calculated on experience in 1963 was virtually the same

Notifications of new cases of active tuberculosis are not greatly different in these three centres, Auckland having the highest, and have been falling consistently and gradually over recent years. The very high rate shown by the Mass Miniature Radiography Unit in Wellington doubtless reflects the selected groups examined. Whereas for tuberculosis the first years of operation revealed considerably more cases than later years, sarcoidosis appears in Wellington to be possibly actually increasing and in Christchurch is not greatly reduced in prevalence.

If figures from Auckland and Christchurch which have comparable rates of tuberculosis as discovered by Mass Miniature Radiography are compared for sarcoidosis, there is an apparent increase in the colder less humid South where there is also strong suggestion that older age groups are affected. Approximately 60 of all cases of sarcoidosis fall in the 20—40 age group.

Only three Maoris have been found with sarcoidosis at M.M.R. Survey — two in Auckland and one in Wellington. In the clinical experience of Dr J B. Mackay Chest Physician, Wellington, the incidence of sarcoidosis in the native race is not noticeably different from that in Caucasians although numbers are too small to be conclusive. Tuberculosis however has prevalence rate which is approximately ten times greater in Maoris than in others.

The population of New Zealand is overwhelmingly of English, Scottish and Irish descent and it is therefore interesting to note that the prevalence rates for sarcoidosis are not greatly dissimilar to rates in England and in other parts of the United Kingdom.

TABLE I

Area Latitude	Auckland 36° S	Wellington 41 S	Christchurch 43 S
Population served by M.M.R. Units	367 000	302 000	493 000
No of Maoris (approx)	18,000 (4.9 %)	8 000 (2 6 %)	5,200 (1 %)
Period	10 years	11 years	7 years
Nos. X-rayed at M.M.R	376,380	306,547	396,353
males	—	191,805	237,873
females	—	114 742	1.8,480
Total no. of cases of sarcoidosis	23	75	73
males	12	44 = 22.9/100 000	32 = 13 45/100 000
females	11	31 = 27 0/100 000	41 = 23.87/100 000
Total no. of cases of sarcoidosis in Maoris	2	1	0
Prevalence rate of sarcoidosis per 100,000			
1st yrs of survey	—	21 1	21.5
1st 6 yrs of survey	—	19.2	—
last 5 yrs of survey	—	8.4	17 6
Overall	6.13	24.3	18.41
Prevalences rate of TB at M.M.R. per 100,000			
1st 2 yrs of survey	—	215	111
last 5 yrs of survey	—	132	51
Overall	72.9	175	68.6
Notified respiratory TB, new cases, 1962 rat per 100,000	57	40	34 7
Ratio TB Sa coidosis (M.M.R.)	12 1	7 1	3.3 1

Population figures are taken from the 1962 Annual Report of th Department of Health and ha been corrected for approximate populations covered by different M.M.R. units.
The same Medical Officers have been in charge for the periods quoted.

TABLE II

Age	Auckland		Wellington		Christ hurch		Totals		Combined Totals	
	M	F	M	F	M	F	M	F		
10—19	1	1	0	1	1	1	2	3	5	2.9
20—29	3	4	20	11	9	10	32	25	57	33 3
30—39	4	2	15	6	9	8	28	16	44	25 7
40—49	2	3	7	7	5	6	14	16	30	17 5
50—59	2	1	1	5	5	8	8	14	22	12 9
60—69	0	0	0	1	3	8	3	9	12	7 0
70	0	0	1	0	0	0	1	0	1	0.6
Totals	12	11	44	31	32	41	88	83	171	

From the Department of Pathology, Allmänna sjukhuset, Malmö, Sweden

The Prevalence of Sarcoidosis in the Autopsy Material from a Swedish Town

I. Hägerstrand and F. Linell

The study was made in the town of Malmö, which has about 250,000 inhabitants. It is situated in the south of Sweden and lies opposite Copenhagen. The population is increasing at the rate of about four to five thousand inhabitants a year, the increase being due mainly to people moving into the town from elsewhere. As far as medical care is concerned Malmö is a single administrative unit. There is only one general hospital in the town. Broadly speaking, only inhabitants of Malmö are admitted to this hospital. The autopsy frequency at the hospital is high (99 %). Most people dying in Malmö do so in hospital with the result that about 60 % of all people who die in Malmö are autopsied at our department. We therefore have an excellent opportunity to study the prevalence and incidence of diseases in a circumscribed population.

The investigation covers the years 1957 to 1962. During this period all autopsies have been made by standardized methods including fairly extensive histological examinations.

Fig. 1 shows a diagram of the age distribution of the material for 1959 only, but it is representative of the entire period.

The high age classes are well represented. People in Malmö get old. The peak in the age distribution was between 75 and 80 years. Incidentally it may be mentioned that nearly 40 % had malignant tumours.

The figures for sarcoidosis are seen in the table.

For comparison the number with active tuberculosis are given. By active means active, mostly florid, tuberculosis with bacilli. More than three fourths of these patients had pulmonary tuberculosis.

It is clear that active tuberculosis is almost three times as common as sarcoidosis. Sarcoidosis is, roughly, equally common in both sexes. We found no demonstrable correlation between sarcoidosis and occupation. It is not always easy to make a firm diagnosis of sar

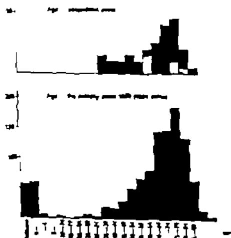


Table I

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females	—	114 744	158,480
Total no. of cases of sarcoidosis	23	75	73
males	12	44 = 22.9/100 000	32 = 13.45/100,000
females	11	31 = 27.0/100 000	41 = 25.87/100,000
Total no. of cases of sarcoidosis in Maoris	2	1	0
Prevalence rate of sarcoidosis per 100 000			
1st 2 yrs of survey	—	21 1	21.5
1st 6 yrs of survey	—	19.2	—
last yrs of survey	—	28.4	17.6
Overall	6.13	24.5	18.41
Prevalence rate of TB at M.M.R. per 100 000			
1st 2 yrs of survey	—	215	111
last 5 yrs of survey	—	132	51
Overall	72.9	173	68.6
Notified respiratory TB, new cases, 1962 rate per 100,000	57	40	34.7
Ratio TB Sarcoidosis (M.M.R.)	1 1	7 1	3.5 1

Population figures are taken from the 1962 *Annual Report of the Department of Health* and have been corrected for approximate populations covered by different M.M.R. units.
The same Medical Officers have been in charge for the periods quoted.

Table II

Age	Auckland M	Auckland F	Wellington M	Wellington F	Christchurch M	Christchurch F	Totals M	Totals F	Combined Totals	
10—19	1	1	0	1	1	1	2	3	5	2.9
20—29	3	4	20	11	9	10	32	25	57	33.3
30—39	4	2	15	6	9	8	28	16	44	25.7
40—49	2	3	7	7	5	6	14	16	30	17.5
50—59	2	1	1	5	5	8	8	14	22	12.9
60—69	0	0	0	1	3	8	3	9	12	7.0
70	0	0	1	0	0	0	1	0	1	0.6
Totals	12	11	44	31	32	41	88	83	171	

From the Department of Pathology Allmänna sjukhuset, Malmö, Sweden

The Prevalence of Sarcoidosis in the Autopsy Material from a Swedish Town

I. Hägerstrand and F. Linell

The study was made in the town of Malmö, which has about 230,000 inhabitants. It is situated in the south of Sweden and lies opposite Copenhagen. The population is increasing at the rate of about four to five thousand inhabitants year the increase being due mainly to people moving into the tow from elsewhere. As far as medical care is concerned Malmö is single administrative unit There is only one general hospital in the town. Broadly speaking, only inhabitants of Malmö are admitted to this hospital. The autopsy frequency at the hospital is high (99 %) Most people dying in Malmo do so in hospital with the result that about 60 % of all people who die in Malmo are autopsied at our department. W therefore have an excellent opportunity to study the prevalence and incidence of diseases in circumscribed population.

The investigation covers the years 1957 to 1962. During this period all autopsies have been made by standardized methods including fairly extensive histological examinations.

Fig. 1 shows diagram of the age distribution of the material for 1959 only but it is representative of the entire period.

The high age classes are well represented. People in Malmö get old. The peak in the age distribution was between 75 and 80 years. Incidentally it may be mentioned that nearly 40 % had malignant tumours.

The figures for sarcoidosis are seen in the table.

For comparison the number with active tuberculosis ar given. By active means ac tiv mostly florid, tuberculosis with bacilli. More than three fourths of these patients had pulmonary tuberculosis.

It is clear that active tuberculosis is almost three times as common as sarcoidosis. Sarcoidosis is, roughly equally common in both sexes. W found no demonstrable correlation between sarcoidosis and occupation. It is not alway easy to make firm diagnosis of sar

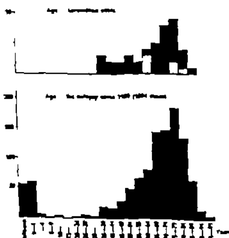


TABLE I

Area Latitude	Auckland 36 S	Wellington 41 S	Christchurch 43 S
Population served by M.M.R. Units	367 000	302,000	493,000
No. of Maoris (approx)	18 000 (4.9 %)	8,000 (2.6)	4,200 (1 %)
Period	10 years	11 years	7 years
Nos. X-rayed at M.M.R.	376,380	306,547	396,353
males	—	191,805	237 873
females	—	114 742	158,480
Total no. of cases of sarcoidosis	23	75	73
males	12	44 = 22.9/100 000	32 = 13.45/100,000
females	11	31 = 27.0/100 000	41 = 25.87/100 000
Total no. of cases of sarcoidosis in Maoris	2	1	0
Prevalence rate of sarcoidosis per 100 000			
1st 2 yrs of survey	—	21 1	21.5
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20—29	3	4	20	11	9	10	32	25	57	33.3
30—39	4	2	15	6	9	8	28	16	44	25.7
40—49	2	3	7	7	5	6	14	16	30	17 5
50—59	2	1	1	5	5	8	8	14	22	12.9
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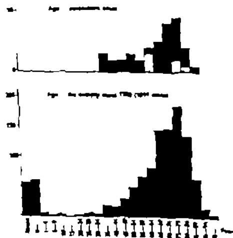


TABLE I

Year	Number of autopsies	"Certain" sarcoidosis Generalized and/or lung sarcoidosis Total	♂	♀	Sarcoidosis regional to malignant tumours	Uncertain cases	Active tuberculosis
1957	940	4	1	3	5	0	18
1958	938	5	3	2	1	2	15
1959	1094	8	2	6	3	4	18
1960	1194	10	5	5	3	7	26
1961	1220	8	3	5	1	4	24
1962	1320	8	4	4	1	7	22
Total	6706	43	20	23	14	4	123
		0.64			0.21 %	0.36 %	1.83 %
						0.57	

coidosis. As criteria of the disease we accepted only cases with so called typical sarcoid granulomas and with wide-spread lesions. The condition was said to be generalized in cases with granulomas in several groups of lymph nodes and/or in the lungs, liver and spleen or other organs. Only cases really satisfying these requirements were accepted as sarcoidosis. Cases that might have been trivial foreign body granulomas, so-called sarcoid reactions and the like were excluded.

We feel that the prevalence of sarcoidosis is in reality higher than that suggested by the figures in the table. But even then I think one may say without fear of contradiction that sarcoidosis is a fairly rare disease (0.64 %). If the uncertain cases in our autopsy series be included, it would bring the figure up to 1 %. There is not made any attempt to discuss the prevalence in our material with that in other and less comparable series. However examination of a Stockholm series (mass chest radiography) suggested a prevalence of about 0.6 per 1000 i.e. less than one tenth of that found in our autopsy material.

From a clinical point of view our cases were not important. In only 3 cases had the disease been diagnosed before death. In only 3 cases could the disease be regarded as the main cause of death. Two patients with generalized sarcoidosis and pulmonary fibrosis died from chronic pulmonary hypertension, and one with myocardial sarcoidosis died from an attack of Adams-Stokes syndrome. In this third case sarcoidosis had not been suspected clinically. In all the other cases in our material sarcoidosis was only a subordinate disease found incidentally in association with the lesions mainly responsible for death. In as many as 22 cases the main disease was malignant tumour. The remaining cases were dominated by cardiovascular diseases. In 40 of the 43 cases the sarcoidosis was an incidental finding, and in retrospect the patient's symptoms could not as a rule be related to the sarcoidosis. On the basis of the present material we therefore feel that sarcoidosis is a much more common disease than what may be supposed from the number of cases diagnosed. Most cases probably run a silent course. The result of our little investigation appears to be worth bearing in mind when judging the figures given for the frequency and causes of sarcoidosis on the basis of epidemiological data collected from clinical departments.

The number of cases diagnosed clinically was less than one tenth of the total number found in our material which however says nothing of the frequency of cases that had been diagnosed clinically and healed completely. The fact that the disease was not men-

tioned in patient clinical history does not exclude the possibility of silent sarcoidosis in youth or middle age which afterwards more or less completely disappeared. It may however be assumed that in many of the cases we saw in old people the lesions had persisted unchanged for many years. Sarcoidosis is, as Uehlinger says, characterized by "reactive conservation.

In fig. 1 the age distribution of the sarcoidosis series is compared with that of the entire material. The age distribution of these two collections does not differ significantly from one another except possibly for small difference in the lower age classes. The shaded columns indicate cases with lesions localized to the lungs and hilus lymph nodes only. We can hardly say that these cases were on the average younger than the remaining cases.

In summary sarcoidosis is not very common disease in our community. But it is seen at autopsy about ten times more often than clinically which suggests that the disease usually runs a silent course and that caution must be exercised in the evaluation of the frequency and causes of the disease, especially of conclusions based only on clinical data.

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Compulsory Notification of Sarcoidosis

From the Swedish National Board of Health, Stockholm

Introduction

ARTHUR ENGEL

Just a few words. As I mentioned yesterday we have thought over if we should try to introduce into this country for a certain period of time a compulsory notification of sarcoidosis in order to get a picture of its distribution geographical, in age groups, and other social groups. I regret very much that I was not present here this morning to listen to your presentations but I have heard from one of the participants that professor Linell presented some observations on the frequency of sarcoidosis in autopsy materials showing a very high incidence of this disease and that most of the cases were not diagnosed during life. So, it is a very vague and difficult thing to introduce such a notification. I would like to ask you if you feel that from the scientific point of view it should be of some interest that one country like Sweden, for some time introduces such a compulsory notification. Would that serve any reasonable purpose?

DISCUSSION

DR LÖFGREN: The suggestion made by Dr Engel for discussion at this conference is very important and interesting. No doubt compulsory notification of sarcoidosis would give more exact information on the occurrence of the disease than we have at present. Consequently I think it would be of great value if an attempt were made to introduce compulsory notification in a few countries with high prevalence, such as Denmark and Sweden, for example, and simultaneously in a few other countries with low prevalence rate. An analysis and comparison of the results after let us say five years, would probably produce much valuable information on the real frequency of sarcoidosis in its different clinical forms.

Is there compulsory notification of sarcoidosis in any country and—in such case—what is your experience of the system?

DR LEVINSKÝ: There is not a compulsory notification of sarcoidosis in Czechoslovakia. But in 79 of 118 districts the patients with pulmonary sarcoidosis, detected by mass photofluorography or in hospitals, are registered in the tuberculous departments of policlinics in the same manner as the tuberculous patients. It would not be difficult in Czechoslovakia to introduce compulsory notification of all forms of sarcoidosis for a limited time as, for example, for five years as proposed by Löfgren.

DR. PUTKONEN: In Finland, all hospitals had to notify the State Medical Board of the diagnosis of each patient under hospital treatment during 1900. Thus we obtained a list of those sarcoidosis patients who had been treated in various parts of the country. It appeared, however from the geographic distribution of the cases and from an

DR POLLAK A review of the autopsy material of the Pathological Institute of McGill University in Montreal shows that during the twenty year period from 1941—1959 eight thousand, seven hundred and forty-one autopsies were performed. (8,741) In twenty cases, evidence of sarcoidosis was found, but in only two cases death could be ascribed to the disease. In three cases, co-existence of tuberculosis and sarcoidosis was noted. In four of the twenty cases, death was due to neoplasm. The others died from a variety of conditions, but all showed sarcoidosis-like granulomata in the lungs and mediastinal lymph nodes.

DR UEHLINGER A valid set of statistics concerning the frequency of sarcoidosis is very difficult to compile when only employing autopsy findings. For it is then impossible to morphologically differentiate from sarcoidosis the endogenous lymphoglandular tuberculous reinfection of elderly patients, which Kudlich and Ghon viewed as essentially a productive non caseating lymphnode tuberculosis with an accompanying rich hyaline exudate. Since it is basically accepted that sarcoidosis may not be definitively diagnosed solely from anatomical findings, excepting the rarer classical cases, I would tend to regard the figures as presented by Linell with a certain degree of caution and prudence.

DR REID I have no statistical information to add but I entirely agree with Dr Linell views on diagnosis. I should like to ask Dr Uehlinger how he distinguishes the old-age form of tuberculosis he describes from sarcoidosis, if this cannot be done histologically and how he knows it is tuberculosis. I imagine that Dr Linell and most pathologists would only diagnose sarcoidosis on autopsy material if cultures were negative for tubercle bacilli. If histological appearances are entirely compatible with sarcoidosis and if cultures are negative, how can one decide that it is tuberculosis?

DR RINGSTED I would like to thank Professor Linell for very interesting paper. As far as I know a similar material has not been examined in Denmark.

DR JÄRVI I am working in Finland and we still have very much tuberculosis in our country. It has been difficult for me to make the diagnosis of sarcoidosis, actually I have never succeeded to do it in autopsy. I have done it from biopsy but I have had some cases where, if one has cut the whole lymph node through, one can find larger necrotic focus in addition. Or in autopsy if one looks at every lymph node, one can find very many which look quite as in sarcoidosis, but one finds one or two larger necrotic nodes somewhere. I want to ask Professor Linell how many histological specimens are taken in this material from every autopsy case and if the autopsy is done so thoroughly that one has really examined every possible gland in the corpse.

DR SILTZBACH I agree with Professor Järvi that it is quite easy to overlook at autopsy old recovered cases of sarcoidosis where there is some other cause of death. Recently Dr Hans Popper our Director of Pathology at the Mount Sinai Hospital, New York, showed me a case in which he found a few hyalinized tubercles in one or two lymph nodes but nothing else grossly impressive pointing to sarcoidosis. When he went through all the organ sections with a fine tooth comb, so to speak, he found hyalinized tubercles in widely scattered organs of the body and even found some tubercles that still retained their epithelioid structure. Quite a few of these were still present in the lung which showed also scarring and emphysema. I am convinced that Professor Linell is not overestimating the incidence of undetected sarcoidosis in his series. Sarcoidosis is far more common, not only in Sweden but in many countries, than we tend to think.

DR NORRIS Iwasaki and Iwai have found in the recent reports or periodicals in Japan thirteen autopsy records of the cases, diagnosed as other than sarcoidosis, however suspected to be the latter disease, e.g. being diagnosed such as Giant cell myocarditis cases. They were allowed to reexamine those cases carefully and disclosed that nine out of these thirteen instances were sarcoidosis cases as suspected. And in five of those cases their causes of death were revealed to be the myocardium sarcoidosis.

DR LINELL My best thanks to all contributing to the discussion. To Dr Uehlinger: As I said, it is always difficult to make a firm diagnosis of sarcoidosis and I agree with him that we can only say that they are cases that give suspicion of sarcoidosis. We have epithelioid granulomas of type and distribution well agreeing with the diagnosis of sarcoidosis. It can be very difficult to differentiate between sarcoidosis and chronic tuberculosis. Here we touch on questions about the definition of sarcoidosis. — To Dr Järvi: We take about 3 blocks of the lungs and we cut the lymph nodes from the hilar region, from the neck, and from the axillar, inguinal and retroperitoneal regions in all cases of malignant tumours and all other cases where we find anything remarkable about the lymph nodes.

performed and yet, at autopsy fungal disease or lymphoma has been found. This need not discourage us if we fully realize that we are dealing with disease which is difficult to diagnose with certainty much more difficult than tuberculosis in this regard where positive tuberculin test or sputum containing tubercle bacilli renders that task relatively easy

Dr Commons: In my view the opportunity for reporting all new cases of sarcoidosis detected during any well defined period of time in well defined population does exist in countries such as Denmark, Sweden and I think in Czechoslovakia as Dr. Levinsky told us. It would appear to me that this is not much different from the early photofluorograph made for the diagnosis of tuberculosis some 20 years ago. W did not have facilities for making cultures and everyone was not doing guinea-pig tests. Yet we got measure of the rate of tuberculous infection in given population. So I would be interested of an attempt at compulsory notification of sarcoidosis suspects being made in certain selected places.

I hav been thinking about how one could persuade public health officials to study the problem that Dr Siltzbach raised, viz. that if you screen large population you come up with large number of suspects. My iew is that these suspects could be further studied using Dr Siltzbach diagnostic pproach to the disease. If w had standardized Kveim antigen this might be added to the culture for tubercle bacilli, for example. So, I would hope that some people would leave this conference giving serious thought to establishing the mechanism whereby standardized detection and registration and follow up could be undertaken.

Dr Evans. I have not ery much to add. I put the question just to get some guidance for my enacting and certainly I got it, and I thank you very much for your contributions to this discussion. And I am most obliged to my colleague Professor Lindell if he would kindly send me his paper which I should like to study. Thank you very much.

analysis of the case records, by Drs. Riska and Selroos, that in Finland the time is not ripe for compulsory notification of sarcoidosis cases. That may be the case in Sweden, but we in Finland have to wait.

Dr Fried In der Bundesrepublik Deutschland ist Anfang 1962 vom „Deutschen Zentralkomitee zur Bekämpfung der Tuberkulose" angeregt worden, alle bekanntwerdenden Fälle von Sarkoidose statistisch zu erfassen. Bisher wurden alle Tuberkulosefälle nach Form und Alter statistisch geführt; jetzt soll zusätzlich eine besondere Rubrik für Sarkoidose eingerichtet werden. Früher haben viele Tuberkulose-Fürsorgestellen die aktive Sarkoidose als Sonderform der Tuberkulose mitgezählt. Die Statistik der Sarkoidose wird auf freiwilliger Basis erfolgen.

Ich möchte glauben, dass diese Statistik noch schlechter ist als die Tuberkulosestatistik. Bei meinen früheren epidemiologischen Untersuchungen konnte ich feststellen, daß in einzelnen Kreisen (in Deutschland gibt es etwa 400) nie ein Sarkoidosefall bewußt beobachtet worden ist. In den Stadtkreisen, Großstädten und in der Nähe von Universitätskliniken sind dagegen relativ zahlreiche Fälle diagnostiziert worden.

Das Ergebnis der neuen Statistik wird abhängig sein von der diagnostischen Intensität und wir können vor Ablauf von 10 Jahren hieraus sicher keine epidemiologischen Aussagen über die Sarkoidose machen.

Dr Linell I don't think that compulsory notification of sarcoidosis is a good way to get a clear and true picture of the frequency of sarcoidosis because there are so many differences in diagnostic tools, knowledge and interest in the disease in different parts of a country and between different countries. I think the best way to get knowledge of the frequency of sarcoidosis is to pick out a few parts in different countries. They should be parts with well-defined population, good hospital and autopsy service. The people making the diagnosis, both clinicians and pathologists, have to come together at meetings and make up the criteria for diagnosis, working methods etc. and then work for one or two years and after that compare their results. I think that will be a WHO project to make such a survey of the frequency of sarcoidosis in different parts of the world.

Dr Löfgren Professor Putkonen and Professor Linell told us that the prevalence figures for sarcoidosis obtained from different regions of the same country may vary widely. I can confirm this by mentioning that one county in Sweden (Jämtland) has a prevalence figure, which is twenty to thirty times higher than the corresponding figures for the counties with the lowest prevalence rates. I do not suppose that this great variation is due to a real difference in the occurrence of sarcoidosis, but is attributable to the fact that the physician responsible for the highest figure has a special interest in and knowledge of sarcoidosis.

Similar points of view may possibly be applied, to some extent, to the variations shown in the world-wide prevalence table presented here today.

It seems to me that the differences in the registered prevalence of sarcoidosis—within a single country or between different countries—are no argument against the introduction of compulsory notification of sarcoidosis. On the contrary, if only we bear in mind that the figures obtained are tentative and, to some extent, unreliable, they can serve to stimulate interest in detecting sarcoidosis and in acquiring a more thorough knowledge of the disease.

Dr Scadding I speak with diffidence on this subject, as I am not personally concerned with the epidemiological aspect of disease, though naturally interested in it. It is my impression that in Great Britain notification of disease is enforced only when some action in relation to the control of that disease will spring directly or indirectly from the notification. Since no practical consequences are likely to follow notification of sarcoidosis, I doubt whether we are likely to persuade anyone in authority to place sarcoidosis on the list of notifiable diseases. I doubt also whether the figure derived from compulsory notification would have much scientific significance, in view of widely differing diagnostic standards and procedures in different areas. The difficulties inherent in securing uniform standards have recently been brought to our notice in a survey of the prevalence and incidence of sarcoidosis in a number of defined areas chosen for their contrasting geographical, industrial and other features, which is being conducted under the auspices of the British Tuberculosis Association. In this survey clinical, radiological and histological evidence about each case is reviewed by a central panel. We think that we have admirable co-operation from physicians, dermatologists, ophthalmologists, pathologists and others in all areas, but it is clear that considerable effort is required to secure reporting of all cases of sarcoidosis in its protean manifestations, even in a small number of well defined areas.

Dr Siltzbach With regard to notification of sarcoidosis, it will have to be remembered that most of the data will come from mass survey X-rays. There is an enormous gap between finding enlarged hilar nodes and/or pulmonary mottling on X-ray and the establishment of a definitive diagnosis of sarcoidosis. Many patients have been thought throughout life as having sarcoidosis after extensive biopsy investigations have been

TABLE I. International Kveim Study Diagnostic Groups Tested 797 Patients

Country	Collaborating investigators	No. of subjects K tested	Biopsy confirmed sarcoidosis	Sarcoidosis suspects	Other diseases granulomatous and non-granulomatous disorders. Controls
England	D. G. James R. Anderson	129	33	47	49
Sweden	S. Löfgren S.-E. Olsson	125	61	42	22
Denmark	O. Horwitz	95	56	34	5
Japan	K. Kitamura et al.	70	26	25	21
Israel	J. Rakower W. Davidson E. Racz	60	21	15	26
Uruguay	P. Purriel	38	29	9	0
W. Germany	K. Wurm I. Zeller	38	21	16	1
France	J. Turiaf	36	14	14	8
Puerto Rico	E. Ramirez E. Figueroa	36	13	12	11
Switzerland	E. Uehlinger R. Wittmer A. Hunziker H. Haas P. Vallbemoz	34	13	14	7
Italy	G. Daddi G. Perman L. Bonomo	25	6	14	5
Hungary	L. Mándi	23	16	4	3
Egypt	A. Sami	19	2	12	5
Australia	T. Hurley B. Ritchie	17	8	4	5
Canada	R. Lane R. Aikens M. Douglas	13	6	6	1
Ireland	B. O'Donnell K. Quinn	12	2	10	0
Finland	T. Putkonen N. Riska	10	5	3	2
Brazil	N. Bethlem	7	4	2	1
Chile	A. Vásquez R. Katz	7	1	6	0
Venezuela	T. Maceda	2	0	1	1
Poland	M. Zeryski	1	0	1	0
	Total	797	317	307	173

3—41364

RESULTS OF KVEIM TESTING

Moderator LOUIS E. SILTZBACH

From the Division of Thoracic Diseases Department of Medicine, The Mount Sinai Hospital, New York, N Y

An International Kveim Test Study[1]

LOUIS E SILTZBACH

This international study of the Kveim reaction was undertaken after the conferees of the second International Conference on Sarcoidosis held in Washington, D C. in June 1960 recommended world wide distribution of a validated and standardized sarcoidal tissue suspension as a diagnostic agent in sarcoidosis.

A series of sarcoidal tissue suspensions which Dr Merrill W Chase of the Rockefeller Institute and I prepared from a single human sarcoidal spleen, had been found by us to possess satisfactory sensitivity and specificity as a test agent in sarcoidosis (1—4) I then undertook to supply our test suspension to 40 investigators in 21 countries, each continent being represented, as well as to physicians in different sections of the United States. In due course 1,228 patients were tested with our suspension—797 patients in those 21 countries and 431 in the United States.

Aims of the International Kveim Trial—The project had a three-fold purpose First, we wished to establish the frequency and level of Kveim responsiveness in various geographic regions among patients having sarcoidosis using a single satisfactory test preparation.

Second, it was thought that our tissue suspension might serve temporarily as a standard against which new preparations of local origin might be calibrated. These locally processed validated Kveim suspensions could then be interchanged to learn the Kveim responsiveness of patients in various countries when test products from several outside sources were employed.

Finally it was hoped that the availability of a reliable Kveim suspension might improve the accuracy of the diagnosis of sarcoidosis and thus add to the meaning of epidemiologic data in this disease. Usually the number of diagnosed subjects increases considerably when the Kveim test supplements the available biopsy procedures.

Along with the vials of our test suspensions,[2] detailed instruction sheets were supplied and the various investigators mailed back to me duplicate slides of the biopsied test sites, as well as forms containing summaries of both the clinical findings and the gross and microscopic Kveim test findings recorded by the local investigator These slides were examined by me and my microscopic readings were then sent to the investigator

[1] This study was supported by Grant AM-02272 from the National Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

[2] Vialing of tissue suspensions was kindly performed by Merck & Co., West Point Pennsylvania.

TABLE III. International Kveim Study-Results and Kveim Tests in 317 Subjects with Diagnosis of Sarcoidosis Confirmed by Organ Biopsy

Category	No. of subjects	% with pos. K	Category	No. of subjects	% with pos. K
Male	156	50	Subacute	158	58
Female	161	50	Chronic (2 yr +)	159	42
Caucasian	271	48	Onset-E. N.	54	35
Negro	20	55	Mass survey	68	40
Yellow	26	64	Respiratory	93	51
			Cutaneous	34	65
Under 40 years	186	55	Other	68	47
Over 40 years	131	43			
Hilar aden.	128	59	Ocular lesions	27	52
Hilar aden. + mottling	110	42	Skin sarcoids	60	60
			Other extrathoracic	69	51
Mottling only	51	43	Negative 100 TU		
Normal X-ray	28	50	Negative 10 TU	159	50
Raised globulin	79	53	*Positive 100 TU*	43	33
Normal globulin	115	50	Positive 1—10 TU	86	52

confirmed group second, 307 or 38 % were sarcoidosis "suspects" with features of clinical sarcoidosis but without organ biopsy support and finally it turned out that 173 subjects or 22 % of those who were Kveim tested did not have sarcoidosis. This category included patients with other granulomatous disorders such as tuberculosis, leprosy, local sarcoid reactions, etc. The technical aspects of the Kveim test will not be considered here since they are described elsewhere (1—3).

Clinical manifestations of sarcoidosis in the various countries. The Kveim study gave us an opportunity to observe some of the clinical variations of sarcoidosis from country to country. For example, how often do patients with sarcoidosis usher in the illness with erythema nodosum? What is the level of the patients' tuberculin sensitivity, what are their *initial chest X-ray patterns, etc.?*

Table II shows the patterns of clinical presentation in the various countries. The table concerns only those subjects with the diagnosis of sarcoidosis confirmed by organ biopsy or positive Kveim test, 429 in number; subjects with negative Kveim reactions and the control subjects are excluded.

Erythema nodosum occurred in 83 patients or 20 %, but this was a particularly variable feature. Sweden, as expected, had the highest proportion of patients with that onset, 43 %, Japan the lowest, only in one patient in 41 or 2 %. There were 29 % of all patients who had respiratory symptoms at the outset. West Germany had the highest frequency for this mode of onset, 74 %, and Puerto Rico the lowest, none. Chest X-rays at the time of testing revealed 48 % with hilar adenopathy alone, 29 % with mottling combined with hilar adenopathy and 15 % mottling only. Eight per cent had normal chest X-rays. This distribution is fairly similar to that which is encountered in most published series. There is a greater than usual incidence of *extrathoracic* manifestation of sarcoidosis. In all, 174 patients or a little more than 4 out of every 10 patients, exhibited such lesions. This high incidence reflects in some degree the large proportion of chronic and symptomatic cases included in this series (43 %). The skin and eyes were involved in 14 % and 9 % respectively. Among the Japanese patients 17 % had ocular disease and in Uruguay 22 % had cutaneous sarcoids.

TABLE II International Kveim Study Clinical Presentation of 429 Subjects with the Diagnosis of Sarcoidosis Confirmed by Organ Biopsy or Positive Kveim Reaction

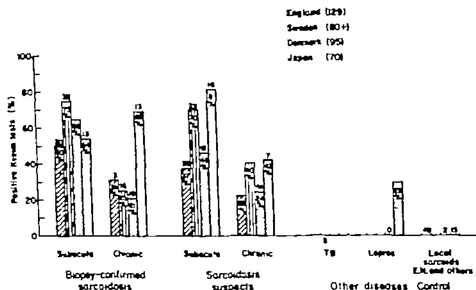
Category	Total frequency No.	%	Countries with highest frequency: Countries	No.		Countries with lowest frequency: Countries	No.	
Onset								
Erythema nodosum	85	20	Sweden	28	43	Japan	1	2
Mass survey	104	24	Denmark	15	29	W Germany	2	7
Respiratory	123	29	W Germany	23	74	Puerto Rico	0	0
Other	117	27	Puerto Rico	12	55	W Germany	0	0
X-ray								
Hilar adenopathy	208	45	Hungary	12	71	Uruguay	7	22
Hilar adenopathy and mottling	124	29	Denmark	22	43	Hungary	2	12
Mottling only	65	15	Switzerland	6	29	Puerto Rico	0	0
Normal	32	8	Puerto Rico	7	31	Sweden	0	0
Organ involvement								
Ocular	37	9	Japan	7	17	Hungary	0	0
Skin	60	14	Uruguay	7	22	Switzerland	0	0
Other extrathoracic	77	18	England	17	36	Sweden	2	3
Tuberculin test								
Negative 100 TU	137	32	Uruguay	18	56	Israel	0	0
Negative 10 TU	71	17	Israel	14	54	Uruguay	0	0
Positive 100 TU	57	13	Sweden	21	32	Australia	0	0
Positive 1—10 TU	109	25	Denmark	25	49	Uruguay	1	3
*Globulin**								
Elevated	100	25	Uruguay	19	59	Italy	1	8
Sex								
Male	200	4	Puerto Rico	[illegible]	91	Switzerland	4	19
Duration								
Chronic	185	43	Uruguay	27	84	Canada	1	10
Age								
> 40 years	166	39	Denmark	29	37	Italy	1	[illegible]

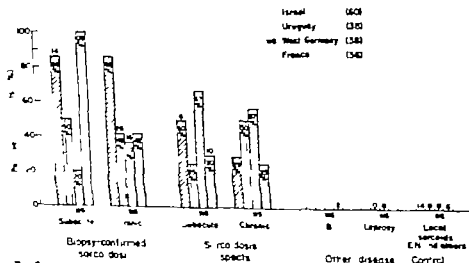
Countries with less than 10 subjects not included.

Countries with less than 33 of subjects tested not included.

Diagnostic groups tested. Table I lists the cooperating investigators from each country as well as the number and types of subjects tested. The countries and investigators are listed in the order of the number of subjects tested 129 and 125 patients were tested by Dr James and Dr Lofgren and their associates, respectively, and in some areas only half a dozen or fewer patients were tested

The three diagnostic groups were constituted as follows first 317 or 40 of the 797 Kveim tested subjects belonged to the biopsy


International Kveim Test Results by Diagnosis and Stage of Sarcoidosis
England (129)
Sweden (80+)
Denmark (95)
Japan (70)
Positive Kveim tests (%)
Subacute
Chronic
Subacute
Chronic
TB
Leprosy
Local sarcoids E.N. and others
Biopsy-confirmed sarcoidosis
Sarcoidosis suspects
Other diseases
Control
Fig. 1


International Kveim Test Result by Diagnosis and Stage of Sarcoidosis
Israel (60)
Uruguay (38)
West Germany (38)
France (34)
Biopsy-confirmed sarcoidosis
Other disease
Control
Fig. 2

The tuberculin test was negative to 10 or 100 units in about half of the subjects. One of four reacted to 1—10 units of tuberculin. These patients thus reveal a somewhat higher incidence of positive tuberculin reactions than do other series. The percentage of males is 47 % and females 53 % The high proportion of males among Puerto Rican subjects is explained by the fact that a good number of those tested were in a Veterans Administration Hospital mainly for men

It would seem from this limited international sample that the clinical manifestations exhibited by patients with sarcoidosis vary considerably from country to country But, as stated, some of these differences can be accounted for by the type of patients available to the investigator for the Kveim test in each country In some countries the proportion of subjects with old lesions was high and in others, quite low Understandably the clinical manifestations are quite different in the subacute and chronic phases of the disease

Results of Kveim test in biopsy-confirmed cases Table III lists results of the Kveim tests in 317 biopsy-confirmed cases of sarcoidosis and the results are based on my own microscopic assessments. One of the many agreeable aspects of the "world wide" Kveim study was the relative infrequency of disagreement between my readings and those of the cooperating investigators in the various countries, many of whom had had little prior experience with the Kveim test. Complete disparity of readings averaged less than 4 incomplete agreement (one of the two readers designated a test as "equivocal") occurred in 8 % more

The point that stands out clearly is that about half of the patients in the various countries exhibited positive Kveim reactions and when subacute cases alone are considered the proportion who were Kveim-positive rises to almost six out of 10 patients, a figure which may be considered encouraging In some countries, the frequency of positive Kveim reactions was high, in fact, similar to that which we have reported from New York City while employing the same suspension (1 3, 4 5) Thus, in Israel the frequency of positive Kveim reactions with our suspensions was 86 % (Fig. 2) in Hungary 69 (Fig 3) and in Japan 62 % (Fig 1) In some countries the frequency of positive Kveim reactions was low West Germany 33 (Fig 2) and Switzerland 31 % (Fig 3) In this early stage of the study it would be unjustifiable to draw too many conclusions regarding the national differences in Kveim responsiveness. Some of the cooperating investigators have told me that with increasing experience they are finding the yield of positive Kveim results rising

Table III shows again that a determining factor in the overall frequency of Kveim responsiveness in this group of biopsy-confirmed cases is the high proportion of patients with chronic illness tested, i.e. half of the entire group. Whereas 58 % of the subjects in the subacute group responded to our Kveim suspensions, 42 of the patients with chronic illness were positive. A similar ratio of positive reactions is observed when age is considered. Thus, 55 of those under 40 years of age yielded positive reactions whereas only 43 % over 40 years responded. Chronic lesions were more frequent in the older age group.

Approximately three of five patients with hilar adenopathy alone were Kveim positive —another result confirming the observation that early-stage patients are more Kveim-responsive. Of interest also are the patients who began their illness with erythema nodosum as well as those with cutaneous sarcoids. The Kveim tests in these categories were positive in 55 % and 65 % respectively Men and women proved equally responsive. Racial distribution of the tested group was not suited to bringing out differences in Kveim reactivity Finally the status of tuberculin reactivity did not appear to have any significant influence of Kveim responsiveness. Recapitulating, we found that five out of ten patients in the biopsy-confirmed group were Kveim-responsive and, if the disease had been estimated to have existed for less than two years, then almost six of ten subjects responded to our suspension. A similar incidence of Kveim responsiveness was noted among 160 biopsy

International Kveim Test Results by Diagnosis and Stage of Sarcoidosis

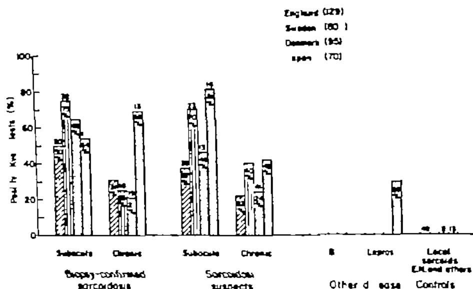


Fig. 1

International Kveim Test Result by Diagnosis and Stage of Sarcoidosis

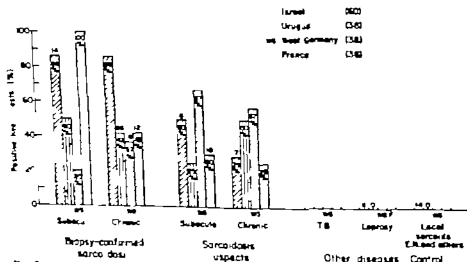


Fig. 2

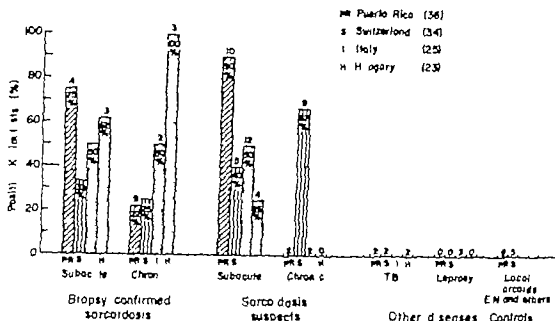


Fig 3

confirmed cases included in a group of 431 subjects tested with our suspensions in arious sections of the United States outside of New York City—fi e out of 10

Results among sarcoidosis suspects" and patients with diseases other than sarcoidosis

Among the sarcoidosis suspects (Fig 1—3 Table IV) we had a combined total of 307 patients and they exhibited a Kveim responsiveness only slightly less than that of the biopsy confirmed group—52 of the subacute and 30 % of the "chronic group of suspects responded to our Kveim suspension

In contrast, among the controls" a combined total of 173 subjects w th tuberculosis, beryllium disease, leprosy local sarcoid re actions and nongranulomatous diseases, only two subjects (1.2 %) both with leprosy ex hibited false-positive rea tions, a satisfac torily low level of mistaken diagnosis. If the international experience parallels the ex perience of the Mount Sinai Hospital and the Rockefeller Institute, then the proportion of such false positive responses with our test suspension will continue to be quite small. These results confirm the specificity of the Kveim test performed with a validated suspension, a specificity that goes beyond that of organ biopsy in the diagnosis of sarcoidosis.

Kveim test results in individual countries The graphs in Fig 1 give the results in England, Sweden, Denmark, and Japan where Kveim tests were performed with our suspension in 419 subjects in all. (Results in Sweden are shown ncompletely in Fig 1 Actually 125 patients were injected in Sweden, and these data are to be given by Dr Olsson)

In almost all countries one notes the consistently higher number of positi K eim reactions among patients in th subacute phase This holds for both biops -confirmed patients and sarcoidosis suspects In Fig 1 under the heading Other Diseases Controls are listed the two false positi reac tions which occurred among seven patients w th leprosy in Japan. No other false positi reactions were encountered among 16 pa tients with leprosy in other countries, nor among patients with any illness other than

Table IV International Kveim Test Results by Diagnosis and Stage of Sarcoidosis

Country	No. of subjects K. tested	Biopsy confirmed sarcoidosis	% K. positive	Sarcoidosis suspects	% K. positive	Other diseases	% K. positive
Egypt	19	2	100	12	25	5 (1 TB)	0
Australia	17	8	50	4	75	5	0
Canada	13	6	67	6	67	1	0
Ireland	12	2	100	10	50	0	0
Finland	10	5	100	3	67	2 (leprosy)	0
Other countries combined[a]	17	5	40	10	10	2 (1 TB)	0

[a] Brazil, Chile, Venezuela and Poland (less than 10 cases).

sarcoidosis. Among the countries in which fewer than 25 subjects were tested (Table IV) is Finland, where there were eight subjects, and among them, five out of five biopsy-confirmed patients and two out of three suspects proved to be Kveim-responsive.

Comment. Taken as a whole the results seem to bear out the idea that the disorder which we call sarcoidosis and observe all over the world is in fact a distinct entity. The frequency of Kveim responsiveness to a single validated test agent in widespread geographic areas supports this hypothesis. The present indications are that any properly screened Kveim suspension can be used to diagnose sarcoidosis everywhere. This interchangeability of test suspensions seems to be incompatible with the supposition that sarcoidosis may be caused by several different immediate inciting agents at different localities in the world.

The present study shows, moreover, that our test suspension produces a good proportion of both large and small Kveim nodules, strong and weak Kveim reactions, among patients having sarcoidosis in all countries. This result indicates cross-reactions are not occurring, for if they were, one would expect to see in one country or another disproportionate predominance of weak or strong Kveim reactions to this single test agent. It seems rather then, that we have recorded widescale specific reaction to an active principle contained in our Kveim suspension.

Were one to consider sarcoidosis to be an atypical form of tuberculosis without demonstrable tubercle bacilli (except rarely), without response to tuberculostatic drugs and without Kveim reactivity, one may then just as readily assume that sarcoidosis might be a special form of beryllium disease without beryllium in the tissues, silicosis without silica, and histoplasmosis without the fungus. The international Kveim trial lends little support to the hypothesis of multiple and geographically varying etiologic agents of sarcoidosis.

Differences from country to country in the frequency of certain clinical manifestations among patients having sarcoidosis have been pointed out in the past and the present study confirms the fact that such variations occur. But similar differences in clinical manifestations are found within the same country. Of greater import, it seems to me, are the differences between all these manifestations of sarcoidosis on the one hand and, on the other the clinical pattern of granulomatous disorders of known etiology such as tuberculosis, beryllium disease and leprosy.

One object of the present study, as mentioned, was to encourage production in bulk

of new standardized Kveim test suspensions employing sarcoidal tissues obtained from patients in various countries. Recently I injected the arm of a patient with five Kveim test suspensions, two of our own splenic suspensions, two lymph nodal suspensions prepared in the conventional manner in England and Finland respectively and one washed splenic suspension prepared in Australia after our Type I prescription. Each of the five suspensions produced a large gross papule with characteristic and almost identical microscopic picture—a gratifying outcome which suggests that soon several validated Kveim suspensions prepared in bulk will be in circulation. For the future, it would seem to be highly desirable to agree upon techniques of processing and screening any Kveim suspensions which may be prepared. We must also pursue standard procedures and criteria for performing and assessing the Kveim test. This cooperative international Kveim trial has been a beginning and augurs well for further international projects in sarcoidosis.

References

1. Siltzbach, L. E. Current Status of Nickerson-Kveim Reaction, Amer Rev Resp. Dis. *84* 89—93 (Part 2) (November) *1961*
2. Chase, M W Preparation and Standardization of Kveim Testing Antigen, Amer Rev *Dis. 84 86—88 (Part 2)* (November) *1961*
3. Siltzbach, L. E. The Kveim Test in Sarcoidosis, JAMA *178* 476—482 1961
4. Hirsch, J G Cohn, Z. A., Morse, S. I Schaedler R. W Siltzbach, L. E., Ellis, J T and Chase, M. W Evaluation of Kveim Reaction as a *Diagnostic* Test for Sarcoidosis, New Eng J of Med. 265, 827—830 (October 26, 1961).
5. Siltzbach, L. E. and *Ehrlich, J. C.* The Nickerson-Kveim Reaction in Sarcoidosis, Amer *J* Med., *16*. *790—803, 1954*

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DISCUSSION

Dr Olsson At Dr Lofgren department (Lung Clinic St. Goran's Hospital Stockholm, Sweden) we have performed the Kveim test for two years in 125 cases with the test suspension used in this international Kveim study Of these, 61 were biopsy confirmed sarcoidosis, 42 sarcoidosis suspects and 22 controls.

In the 22 control cases there was no false positive Kveim test. In the 103 sarcoidosis cases the results of the Kveim test were positive in 46 with almost the same frequency in the biopsy confirmed group as in the suspects 47 and 45 respectively These average figures, however are incomplete and depend on the character of the material This has been analysed further (Table I and II)

In the group of pulmonary infiltration cases (Table I) 13 cases showed only minimal pulmonary infiltration and 8 out of these 13 cases (62 showed Kveim-positivity This is in the same range as in the group of bilateral hilar adenopathy alone. The remaining 4 cases had more extensive pulmonary infiltration and only 25 positive Kveim tests, which is more close to the fibrotic group. The results demonstrate the higher Kveim responsiveness in early stages of sarcoidosis. They also stress the impossibility of comparing results of Kveim tests without characterizing the patient material tested.

When working with the Kveim test one should be aware of the importance of careful technique We have the impression that the more experience we have gained the more positive tests have been obtained.

One disadvantage of the Kveim test is that the Kveim nodule must be excised. Beautiful young ladies — and sarcoidosis patients very often are will be afraid of an ugly scar In our material we have only a few cases of ugly scars, especially since we started to make punch biopsies Of course it would be better if one could make the Kveim test elsewhere than on the forearm The thigh might be a good place In order to test whether the skin reactivity is equally good on the

TABLE I. Frequency of positive Kveim tests in different stages of the disease in 103 cases of biopsy confirmed sarcoidosis and sarcoidosis suspects

	Number of cases Kveim tested	Percentage Kveim tests positive
Bilateral hilar adenopathy with erythema nodosum	22	82 %
Bilateral hilar adenopathy alone	23	57 %
Pulmonary infiltration	37	38
Pulmonary fibrosis	18	11 %
Chest X-ray normal	3	(33 %)

TABLE II. Frequency of positive Kveim tests related to the duration of the disease in 103 cases of biopsy confirmed sarcoidosis and sarcoidosis suspects

Duration of the disease	Number of cases Kveim tested	Percentage Kveim tests positive
Less than 2 years	42	81 %
More than 2 years	30	30 %
Unknown	31	13 %

thigh, we made the Kveim test simultaneously on the forearm and the thigh, about 15 cm (6 inches) above the wrist and the knee respectively. In 34 out of 37 cases the results were identical. In one of the remaining 3 cases there was a very weak positive reaction on the arm and negative on the leg, and in 2 cases there was a weak positive reaction on the leg and negative on the arm. Our conclusion is, that one can make the Kveim test equally well on the thigh as on the forearm. The histological picture of the Kveim nodule on the forearm is a little more beautiful because of the thinner skin there, but on the other hand the thicker skin on the thigh makes it a little easier to inject the Kveim suspension more exactly intracutaneously.

Dr Yonaga: The joint world-wide survey of sarcoidosis, applying the same Kveim antigen provided by Dr Siltzbach has really been an epoch-making undertaking. I had been informed that there had been a time, when some of the European specialists in sarcoidosis had doubted, if the so-called sarcoidosis cases described in America had been true cases or not, because of the absence or the rarity of Erythema nodosum there, while it had been a very common manifestation of sarcoidosis cases in Europe. At the time of the Washington Conference, as we had not seen any case of sarcoidosis with erythema nodosum, I have had to lay my stress to convince the conferees that we had been dealing with the same sarcoidosis cases in Japan, as those in America. However now by the merit of the joint world-wide survey of sarcoidosis with Dr Siltzbach Kveim antigen, unequivocal conclusion was made that the sarcoidosis in the participating countries in this survey was an identical entity. I would like to congratulate Dr Siltzbach for such significant merit.

Taking advantage of this opportunity I would like to ask you, Dr Siltzbach, if steroid therapy would be applied to a patient during the course of Kveim testing, would it affect the results of the test or not?

Dr Siltzbach: Professor Nobechi, we are now studying, in a controlled fashion, the effects of corticosteroid therapy on the Kveim reaction. Thus far we have found that the corticosteroid therapy does diminish the exuberance of a Kveim test and may even abolish it completely. However we have a good number of patients where positive Kveim tests have been evoked while the patient was receiving therapeutic doses of corticosteroids.

Dr James: One great snag about the Kveim test is that there is a world scarcity of Kveim antigen. This is partially overcome by Dr Louis Siltzbach generosity. The question facing us is how to overcome the shortage. It seems to me that there is a choice of one of three approaches. *The first* is by regarding the Kveim test as useless and thereby not having to bother about it. *The second* avenue of approach is to endeavour to find a synthetic alternative to Kveim antigen. This is what we have tried to do in our studies at the Royal Northern Hospital, London. We have tried to reproduce the Kveim test with Dr Nine Choucroun tuberculo-lipopolysaccharide, mycolic acids, pine pollen, beryllium, and material from chalazions (see "The Kveim Test in Sarcoidosis", Lancet 1963, 2, 650). Thus far none of these antigens has been as specific or as frequently positive as sarcoid tissue antigen. *The third* avenue is to recognise that Kveim antigen is scarce and to use it sparingly. For instance, I cannot quite see why dermatologists find it of practical value since they presumably can perform a biopsy of the skin lesions. They would of course find the Kveim test of value in distinguishing various causes of erythema nodosum.

of new standardized Kveim test suspensions employing sarcoidal tissues obtained from patients in various countries. Recently I injected the arm of a patient with five Kveim test suspensions, two of our own splenic suspensions, two lymph nodal suspensions prepared in the conventional manner in England and Finland respectively and one washed splenic suspension prepared in Australia after our Type I prescription. Each of the five suspensions produced a large gross papule with characteristic and almost identical microscopic picture—a gratifying outcome which suggests that soon several validated Kveim suspensions prepared in bulk will be in circulation. For the future, it would seem to be highly desirable to agree upon techniques of processing and screening any Kveim suspensions which may be prepared. We must also pursue standard procedures and criteria for performing and assessing the Kveim test. This cooperative international Kveim trial has been a beginning and augurs well for further international projects in sarcoidosis.

References

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2 Chase, M. W. Preparation and Standardization of Kveim Testing Antigen, Amer Rev Dis. 84 86—88 (Part) (November) 1961

3. Siltzbach, L. E. The Kveim Test in Sarcoidosis, JAMA 1 8, 4 6—48, 1961

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5. Siltzbach, L. E. and Ehrlich, J. C. The Nickerson-Kveim Reaction in Sarcoidosis, Amer J Med., 16, 790—803, 1954.

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TABLE I. Results of Kveim test in active sarcoidosis

Test substance	Number of patients	Positive results	
		Number	Per Cent
I	24	16	67 %
II	13	10	77 %

TABLE II. Results of Kveim test in conditions other than sarcoidosis

Condition	Number of patients	Number of tests	Positive Results
Active pulmonary tuberculosis	22	22	0
Other diseases	52	62	0
Normal	1	1	0
Totals	75	85	0

us reactions occur. The unspecific early reactions disappear in a few days. The potency of the Type I antigen is also sufficient. At least 3 of our own antigens were weaker than it, and only 2 were stronger.

Dr Douglas J. Edinburgh, in common with most other experience, we found that Kveim test substances made from sarcoid glands varied considerably in potency and it was only at the third time of trying that a dependable tissue suspension was obtained, some 2 1/2 years ago. Since then we have had experience of three potent evaluated test substances, two of which were produced in sufficient quantities to allow their use in attempting to determine the specificity of the response. Because of our relatively short experience the number of tests we have to record is few compared with other series. To my knowledge, however, there are no previously recorded observations from Scotland on the Kveim test employing locally prepared test substances.

I want to discuss our experience with the Kveim test under three headings: Firstly the positivity rate with the two test substances in cases of active sarcoidosis, secondly the results with the test in a variety of conditions other than sarcoidosis and finally a method of preparation of the test material which may increase the availability of the test.

The first of the test substances and the one produced in the greatest amount (I) gave 16 positive results in 24 patients in whom clinical and or radiographic features allowed a confident clinical diagnosis of sarcoidosis (Table 1). This is a positivity rate of only 67 %, but as the last three tests in the series were negative in patients with florid sarcoidosis it is possible that the test material had, as is known to occur suddenly lost its potency. If this is the case the figure of 16 out of 21 positive results (or 76 %) would be more in keeping with general experience with the test and would approximate to the positivity rate obtained with the second of the test substances (II) which gave positive results in 10 of 13 cases (77 %). In this series biopsy was made at four to six weeks only if a nodule could be palpated at the injection site, and a positive test was recorded only if follicles of epithelioid cells, usually with Langhans giant cells, were present.

All but 5 of the cases tested belonged to the sub-acute group with hilar glands with or without associated pulmonary opacities. Two patients had persisting hilar glandular enlargement over seven years with no other developments apart from cervical lymphadenopathy in one which afforded further histological proof of the diagnosis. In one patient where intestinal resection had demonstrated histology compatible with sarcoidosis a positive result was obtained with both test substances. One patient previously diagnosed as pulmonary tuberculosis but without bacteriological confirmation presented later with bizarre radiographic appearances of the lungs, which seemed more compatible with tuberculosis, but a positive Kveim test was demonstrated with the two test substances, and radiography of the hands and feet showed appearances compatible with the cystic osteitis of sarcoidosis. One patient with lupus pernio also gave a positive result with both substances.

When the second of the test substances became available, most commonly both were employed simultaneously in the same patient. In one patient there was a negative result with one and positive with the other and the reverse order of positivity was seen in another patient. It may be that this was due to faulty technique and that insufficient particulate material was injected in the negative tests. Unfortunately patients with the features of active sarcoidosis but with initially negative Kveim tests were not re-tested in this series.

In 4 cases biopsy was made of a palpable nodule which developed at the site of injection, but no specific histology was demonstrated and these were, of course, considered negative.

Seventy-four patients with a great variety of pathology (including 22 with active tuberculosis) and one normal were tested with test substance I, and in 10 of these test substance II was also used. In 85 tests no palpable nodule developed which

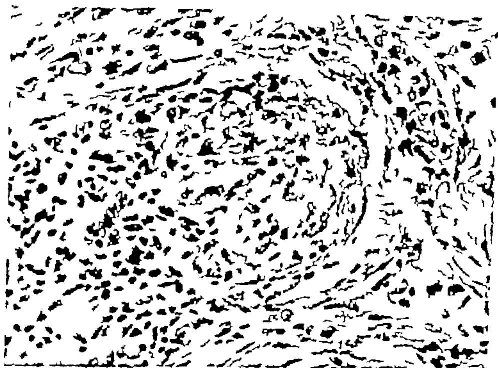


Fig. 1. Positive Kveim reaction. Excision 34 days after inoculation. K. Klara, 47 years. MB 11378/62 200 1 (Uehlinger see below)

Finally what of th future of the K eim test. *If there was sufficient antigen vailable then t* might probably be applied t assessing world prevalence of sarcoidosis, *perhaps in conjunction* with mass radiography of the chest.

Dr Si tzbach It would be *most useful* t widen the application of the K eim test and use it in combination w th mass *radiography. In New York City today at the Mount Sinai Hospital* and the Rockefeller Inst tute Sarcoidosis Clinics, large majority of the patie ts are referred by local public health clinics where survey film has disclosed findings on the chest roentgenogram suggestive of sarcoidosis. Th first diagnostic measure which is applied to these subjects when they appea t the Sarcoidosis *Clinic is the K eim* test nd often that is ll that is necessary. What interests me now is early diagnosis of sarcoidosis, perhaps before clinical evidence ppears. It strikes me that one might hoose small geographic area of high incidence of sarcoidosis and pply the Kveim test to the general population in th 20—40 ge group. One could then learn whether the disease can be detected even *before* hilar adenopathy or erthyema nodosum becomes manifest. For this, one needs large amounts of validated Kveim material and many hands in a functioning organization.

Would Professor Uehlinger say that the dermatologist may have difficulty distinguishing between cutaneous sarcoids and the lesions of other granulomatous skin disorders? I believe that the Kveim test might be of aid as well in distinguishing between these conditions.

Dr Lüthi I ha e enjoyed much success in evaluating K eim reactions by strictly adhering t the principles proposed by Siltzbach nd therefore limiting m interpretations t positi , nega ti e, or normal. The interpretation is ery easy when no reaction takes place. Similarly easy t interpret are the typically positive reactions ith epitheloid cell nodes or the negative reactions showing perivascular histiocytic excrescences Fig 1. However difficulty arises when attempting t evaluate subepithelial band-like histiocytic formations around stratified serous exudation, or the incomplet reactions ppearing during continued cortisone therapy. Yet of paramount significance in allowing for the proper appraisal of K eim test is the quality of the employed antigen. I ha found that the Chase-Siltzbach antigen gives he finest results. Therefore if the proper antigen is used, th K eim Test may be considered one of the most valuable criteria for the diagnosis of sarcoidosis.

Dr Putkonen W hav been ery happy, Dr Siltzba h, to ha the opportunity of comparing our ant gens with your antigen Type I which seems to be so highl purified that no false posi

SKIN REACTIVITY IN SARCOIDOSIS

Moderator Anand Meyer

From the Institute of Diseases of the Chest, Brompton, London, Great Britain

Skin Reactions of Delayed Type

J G Scadding

It is generally agreed that patients with sarcoidosis, as a group, show less reactivity to agents causing delayed skin reactions than do control groups drawn from the same populations. Tuberculin is, of course, the antigen most widely investigated. Depression of skin sensitivity to other agents causing delayed skin reactions has also been reported for instance to trichophytin, the antigen prepared from the fungus causing the common dermal infection to mumps virus to pertussis vaccine, as reported by *Sones and Israel in 1954* to an antigen prepared from Candida albicans (Friou, 1952) The latter observation was confirmed at the Brompton Hospital by Citron (1957) who found that 90 % of control subjects gave reactions to an intradermal injection of an antigen prepared from Candida albicans, but only 40 % of a large series of sarcoid patients gave a reaction. Thus, the generally diminished reactivity of the skin of patients with sarcoidosis to a variety of agents causing delayed reactions is firmly established.

On the other hand, the reactivity of patients with sarcoidosis to agents stimulating the production of humoral antibodies and immediate type skin reactions is much less abnormal. They are as liable as anyone else to asthma, hay fever eczema and urticaria. Out of a series of 275 patients with sarcoidosis, there were 3 who had asthma at the time when I saw them, one also had hay fever and one also had eczema, and there were 3 who had had asthma in the past. That patients with sarcoidosis produce circulating antibodies to various antigenic stimuli normally or nearly normally has been confirmed by a number of people. Sones and Israel showed that they produce a normal response to pertussis vaccine, and Sands, Palmer Mayock and Creger (1955) showed that they produce a rather higher titre of iso-agglutinins to small injections of mismatched blood than *do normal subjects.* On the other hand, Greenwood, Smellie, Barr and Cunliffe (1958) found that in sarcoid patients the titre of antibodies produced to primary vaccination with tetanus toxoid was rather lower than in controls, but the titre produced in response to revaccination was rather higher

There are three possible explanations for *the general depression of reactivity of the skin* to agents causing delayed reactions.

One is that in patients with sarcoidosis there may be some specific substance which prevents the development of cutaneous reactions — so-called anticutin

The second is that for some reason the skin in sarcoid patients becomes non-reactive, while other tissues remain normally reactive.

The third is that in sarcoidosis there is depressed production of the sort of antibodies that subserve *delayed type reactions* and are contained in circulating cells.

would justify biopsy (Table II) So far then, no false positives have been found with either test substance. Experience in Edinburgh can, therefore, support the claim that the Kveim test is highly specific for active sarcoidosis, although positi es may be obtained in only bout 3 out of 4 cases w th the best test substances t present available

Although there is usually little doubt clinically about the diagnosis of the great majority of cases of sarcoidosis and, therefore, the Kveim test in these is often something of a refinement as far as diagnosis is concerned there will always be a place for its use in the less obvious case and its role as a valuable tool for study of the immunology of the disease is likely to continue. The scarcity of the test material must always limit the general availability of the Kveim test, but any method which would increase the keeping qualities and the transportability of the test substance would be useful. These were the considerations which prompted a trial of freeze-dried test material made up n individual ampoules containing the solid material from 0.1 ml or 0.2 ml of a fluid suspension (1) These were reconstit ted w th saline and tested in 7 patients who gave positive results with fluid Kveim re-agent I The freeze-dried preparation, which had been kept at room temperature for periods p to ine months, gave equally arked macroscopic reactions as the fluid substance and showed equally typical sarcoid histology In 2 patients the K eim reaction with fluid and freeze-dried substances was positive in spite of continued treatment with prednisolone in dosage of 10 mg and 15 mg per day respectively

In 6 patients w th presumptive sarcoidosis parallel testing of fluid and freeze-dried test substance gave no positive reaction t either and in further 6 patients with arious non-sarcoid pulmonary pathologies no posit ve result was obtained w th the freeze-dried preparation So far then, no false positi es ha been obtained w th the freeze-dried material.

It is too early t make any claims for freeze-dried K eim test substance, but this preliminary experience suggests that more extensive trial might be useful.

Dr Villar I w t t point out the incon enience of the tardiness of the react on. Some of the Portuguese cases did not return for the reading t the end of 4 weeks.

Dr Israel I ha e tw comments. First, your slides specify that the diagn sis in the cases tested was proved by biopsy or by the K eim test If your survey is intended as a measure of the frequency of Kveim reactions in sarcoidosis patients in various parts of the world, you cannot properly include in the study patients whose only histologic evidence of sarcoidosis is the positi K eim test. I think that you should indicate the results of Kveim tests in patients in whom the diagnosis was established by other criteria.

Secondly the studies reported this afternoon seem to indicate that th K eim test is positi in approximately 60 per cent of patients w th sarcoidosis. This represents a considerable reduction from the 85 per cent frequency which I believe you reported at the conference in 1960 I t possible that your antigen is losing potency

Dr Siltzbach In answer to your first question, Dr Israel w did separate the biopsy-confirmed cases from the sarcoidosis suspects. Unfortunately I was not ble to show you all of the slides of the K eim results from the various countries but when these proceedings are published you will note that the separation that you inquired about has been followed. With regard to your second point, the incidence of positi Kveim result in the International Kveim Study being somewhat lower than that which I reported in Washington three years ago, I do not think that the lower incidence can be ascribed to a loss of potency of the Kveim material which w distributed to the various countries. You may recall that in my presentation yesterday "The Nature, Significance, nd Interpretation of the Kveim Reaction the incidence of positive K eim reactions among biopsy-confirmed cases of sarcoidosis at the Mount Sinai Hospital was given as 83 per cent as compared w th the figure of 84 per cent which I reported in Washington in 1960 based on a lesser number of subjects tested. The lower incidence of positive K eim reactions in the biopsy-confirmed group of the subjects in the International K eim Study can be explained, I feel, by two circumstances first, a number of th collabora ing investigators recalled for Kveim testing many know cases of sarcoidosis w th chronic or inacti disease and thus tended to lower the frequency of positi K eim reactions. A second factor is th lack of familiarity on the part of some in estigators with all the details of the K eim test itself As ou heard, Dr Olsson of Dr Lofgren linic reported that he is now getting higher incidence of positive reactions as h becomes more familiar with the finer points of testing and biopsy But I regard even 50 per cen positi K eim reactions as good beginning for the International Study

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There are three possible explanations for the general depression of *reactivity of the skin* to agents causing delayed reactions.

One is that in patients with sarcoidosis there may be some specific substance which prevents the development of cutaneous reactions — a so-called anticutin.

The second is that for some reason the skin in sarcoid patients becomes non-reactive, while other tissues remain normally reactive.

The third is that in sarcoidosis there is depressed production of the sort of antibodies that subserve delayed type reactions and are contained in circulating cells.

I will now consider these three hypotheses.

"Anticutins" Wells and Wylie (1949) and Stirling (1950) found that if tuberculin is mixed with serum from patients with sarcoidosis and used for a tuberculin test in a known tuberculin-positive patient, the reaction appears in some instances to be inhibited. They suggested that this was due to a specific inhibitory substance or anticutin that was present in the serum of the sarcoid patients. This matter was investigated in greater detail by Magnusson (1956) He found that when this sort of test was performed, in some instances a wealing reaction occurred shortly after the injection and that the inhibition of the tuberculin test was related to the appearance of these wealing reactions. Such a phenomenon is well recognised any wealing reaction will inhibit or reduce the intensity of a later tuberculin reaction (Pepys, 1955) Thus the evidence suggesting the existence of anticutins is explicable on other and more general grounds.

Intrinsic reactivity of the skin in sarcoidosis The suggestion that the skin of sarcoid patients might be intrinsically non-reactive was disproved by the investigations of Urbach, Sones and Israel (1952) They showed that tuberculin sensitivity could be transferred to the skin of tuberculin-negative sarcoid patients by intradermal injection of leucocytes from a tuberculin-positive subject, a method by which tuberculin sensitivity can be passively transferred to the skin of a normal tuberculin-negative subject. It is clear therefore that the skin of the sarcoid patients is not intrinsically non-reactive. Further evidence on this point will be given later in discussion of the non-reactivity of the skin which is observed in patients with reticuloses and comparison of this with that observed in sarcoid patients.

Production of cell-borne antibodies There remains for consideration the possibility that in sarcoidosis there is deficient production of the cell borne antibodies which underlie delayed skin reactions. Here again the investigations of Urbach, Sones and Israel are relevant. They showed that skin sensitivity to tuberculin could not be produced in normal tuberculin-negative subjects with cells from sarcoid patients even though some of their sarcoid patients presumably must have had tuberculous infections in the past, none of them were carrying antibodies in their cells. A further study relevant to this point was carried out at Brompton Hospital by my colleague, K. M. Citron (1958) He showed that tuberculin had a cytotoxic effect on leucocytes from patients with pulmonary tuberculosis and from tuberculin positive normal subjects, but not on those from tuberculin-negative normal subjects. In none of the tuberculin-negative sarcoid patients were the leucocytes affected by tuberculin. Since some of the latter patients showed evidence of having had tuberculous infections in the past, this suggested deficient production of cell-borne antibodies.

Aetiological significance of depression of delayed type sensitivity

The depression of delayed-type skin reactivity can be interpreted in different ways to make it compatible with two opposed views of aetiology

On one view the depression of delayed-type sensitiv ty is regarded as one of the effects of sarcoidosis t is thought that some agent at present unidentified causes sarcoidosis, and this same agent or the process to which it gi es rise in the body causes the deficiency of tuberculin-type antibody production.

The other view is that the depressed reactivity to tuberculin-like antigens is part of an immunological state, which is in a sense causal and it is postulated that sarcoidosis occurs as a response to an agent or agents, possibl already known in some other context, in certain individuals who for some reason not t present understood are in a peculiar immunological state.

It is well recognised that in Hodgkin's disease sensitivity to agents producing dela ed skin reactions including tuberculin, is considerably reduced (Steiner 1934 Schier 1954) Hoyle, Dawson and Mather (1954) found that the proportions of their cases of Hodgkin's disease and of sarcoidosis which

reacted to tuberculin were similar and both considerably lower than in control series. But this numerical similarity does not necessarily imply similarity of mechanism, and it is important in assessing the aetiological significance of depression of delayed reactivity in sarcoidosis to determine whether the mechanism of this depression is in fact the same in Hodgkin disease as in sarcoidosis. Some observations relevant to this point are available.

In 1952, we observed that 50 % of tuberculin-negative sarcoid patients would react to tuberculin mixed with cortisone, to give a reaction similar to normal positive tuberculin test (Pyke and Scadding, 1952). We later confirmed these findings in a larger series of patients with sarcoidosis and compared them with the findings in patients with pulmonary tuberculosis, together with control groups (Citron and Scadding, 1957). Tuberculin reactions in tuberculous patients who were highly sensitive, producing reactions to 1 T U were nearly all substantially inhibited by cortisone. In those who were less sensitive, requiring larger doses of tuberculin to produce reaction, the degree of inhibition became less. In few patients who, in spite of having active pulmonary tuberculosis, reacted only to 100 T U the reaction was not inhibited at all by cortisone. A clear pattern thus became apparent. The degree to which tuberculin reactions in patients with tuberculosis were inhibited by cortisone varied directly with the level of tuberculin sensitivity. About this time we were desensitising few patients with pulmonary tuberculosis to tuberculin by graded doses of tuberculin subcutaneously under the cover of antibacterial drugs. Sixteen patients who had been so desensitised, so that the skin no longer reacted to 100 T U were tested with cortisone plus tuberculin eleven of them produced positive reactions, like 50 per cent of the tuberculin-negative sarcoidosis patients. A control series of healthy tuberculin-negative subjects produced no reactions to tuberculin plus cortisone. Thus the tuberculin-negative sarcoidosis patients behaved more like desensitised tuberculosis patients than like tuberculin-negative healthy subjects.

Fairley and Matthias (1960) later investigated patients with Hodgkin disease and reticuloses in the same way and found that only 8 per cent of 62 tuberculin-negative patients with reticuloses produced reactions when cortisone was added to tuberculin in skin test. Thus patients with reticuloses behave differently from sarcoidosis patients in respect of this test.

Further evidence of difference in mechanism between the diminished sensitivity to delayed skin reactions in Hodgkin disease and in sarcoidosis is available. As already noted, Urbach, Sones and Israel (1952) succeeded in transferring tuberculin-sensitivity to tuberculin-negative patients with sarcoidosis by injecting into their skins cells from tuberculin-positive subjects. More recently Kelly Lamb, Varco and Good (1960) and Warwick, Archer Kelly and Page (1961) reported that they had failed to transfer sensitivity of tuberculin type to Hodgkin disease patients even after what they called massive transfer of cells from as many as 5 donors. Thus we may say that the low skin sensitivity of the skin to tuberculin in patients with sarcoidosis is more like that of desensitised tuberculosis patients than either that of healthy tuberculin-negative subjects or that of patients with reticuloses.

The study of the immunological peculiarities of patients with sarcoidosis, among which reduced ability to produce delayed skin reactions is prominent, are likely to be as important as search for external causative agents in the solution of the difficult problems of aetiology. The studies summarised in this paper, though inconclusive, fa our the view that sarcoidosis arises in certain individuals who are in an appropriate immunological state, probably in response to some external causative agent. If this is so, we must allow for the possibility that *there may be several* agents which can precipitate sarcoidosis in susceptible subject, and that observations relating sarcoidosis to external agents in one community may not be applicable to others with different prevalences of possible inciting agents.

References

Citron, K. M. (1957) Skin tests in sarcoidosis. Tubercle, Lond., 38 33.

Citron, K. M. and Scadding, J G (1957) The effect of cortisone upon the eaction of the skin to tuberculin in tuberculous and in sarcoidosis. Quart. J Med. 26 277

Citron, K. M. (1958) Tissue culture studies of tuberculin sensitivity in man. Tubercle, Lond. 39 65.

Fairley G H. and Matthias, J Q (1960) Cortisone and skin sensitivity to tuberculin in reticuloses. Brit. med. J 2 433.

Friou, G J : (1952) Delayed cutaneous sensitivity in sarcoidosis. J clin. Invest. 31 630.

Greenwood, R., Smelhe, H. Barr M. nd Cunliffe, A. C. (1958) Circulating ntibodies in sarcoidosis. Brit. med. J 1 1,88.

Hoyle, C., Dawson, J and Mather G (1954) Skin sensitiv ty in sarcoidosis. Lancet 2. 164

Kelly W D., Lamb, D L., Varco, R. L. and Good R. A. (1960) An investigation of Hodgkin s disease w th respect to the problem of homotransplantation. Ann. N Y Acad. Sci. 87 187

Magnusson, B. (1956) The effect of sarcoidosis sera on the tuberculin response. Acta derm. venereol. 36. suppl. 35.

Pepys, J (1955) The relationship of nonspecific and specific factors i the tuberculin reaction. Amer Rev Tuberc 71 49

Pyke, D A. and Scadding, J G (1952) Effect of cortisone upon skin sensitivity to tuberculin in sarcoidosis. Brit. med. J * 1126.

Sands, J H., Palmer P P Mayock, R. L. and Creger W P (1955) Evidence for serologic hyper-reactivity in sarcoidosis. Amer J Med. 19 401

Schier W W (1954) Cutaneous anergy and Hodgkin s disease. New Engl. J Med. 250. 353.

Steiner P E. (1934) Etiology of Hodgkin disease skin reactions to vian and human tuberculin proteins in Hodgkin disease. Arch. intern. Med. 54 11

Sones, M. and Israel, H. L. (1954) Altered immunologic reaction in sarcoidosis. Ann. intern. Med. 40. 260.

Stirling K. (1950) Erythema nodosum with tuberculin-neutralizing serum. Amer Rev Tuberc. 62. 112.

Urbach, F., Sones, M. and Israel, H. L. (1952) Passive transfer of tuberculin sensitivity to patients w th sarcoidosis. New Engl. J Med. 47 794

Warwick, W J Archer O Kelly W D. and P ge, A. R. (1961) Anergy of delayed allergy in Hodgkin disease patients. Fed. Proc. 20. 18.

Wells, A. Q. and Wylie, J A. H. (1949) Tuberculin neutralising factor in serum of patients with sarcoidosis. Lancet 1 439

From the Sanatorium of Braunwald, Switzerland.

Primary and Secondary Anergy in Sarcoidosis

Erwin Sommer

It is well-established fact that tuberculin reaction in sarcoidosis is weakly positiv or completely negativ in most of the cases. Frlou, Sones and Israel, and other investigators pointed out that in sarcoidosis there is, in general, decrease of the skin reaction to various antigens such as Oidiomycin, Mumps-virus, Pertussis antigen, Trichophytin and so forth. Furthermore, Hempel and Lauterbach showed that in children admitted to children hospital, the tuberculin reaction was decreased in nearly all the diseases requiring hospitalisation. The reaction reached its original intensity only after period of approximately 3 to 7 months.

These authors consider that the reduction of tuberculin sensitivity in sarcoidosis is non-specific phenomenon, without immunologic or etiologic significance.

On the other hand, the supporters of "tuberculous etiology (Fream, Heilmeier, Reindell, Stachmer, Wurm, and others) believe that here specific process is involved, an expression of particular immunity to tuberculosis which they term "positiv anergy".

With Shakespeare one may ask "to be or not to be specific depression of skin reaction in sarcoidosis, that is the question. The reply to this question will also answer the *main question, whether sarcoidosis is* special form of tuberculosis or disease sui generis. For these reasons, during recent years, we have thoroughly and repeatedly tested the intensity and variations in the tuberculin reaction in each one of our patients.

At first we used, in each individual case, aqueous tuberculin. The initial dose was *10 U if necessary it was increased up to* 5,000 U corresponding to 0.1 ml of dilution 1 : 1. With this procedure we obtained positive reaction in about 20 % of all patients. In *the remaining 80 we used depot-tuber*culin in oil, prepared according to Seeberg and James. We then obtained positive reaction in an additional 20 % of all our cases. In *84 cases of sarcoidosis, distributed according* to age, we obtained the following results (Tab. I).

The results of the tuberculin test in sarcoidosis show that the younger the subject, the higher the percentage of negative reactions. This is the same situation as that observed in regard to the tuberculin reaction in healthy population. This result supports the view that only those patients with sarcoidosis will show a positiv tuberculin reaction, who were tuberculin positiv already before their illness. We therefore tried to ascertain which of our patients were tuberculin positiv already before they contracted sarcoidosis. We examined

1) the clinical records
2) the results of previous reactions during school-age or military service
3) *BCG-scars*
4) pulmonary and hilar calcifications of primary complexes. In approximately 30 of our cases real tuberculous calcifications could be demonstrated by tomographic examination of the hili. If we divide our cases into two groups according

TABLE I

Age in years	Positive reaction aqueous or oily tuberculin percentage
10—20	14
20—30	36
30—40	48
40—50	60
50—60	75

to the positive or negative reaction to tuberculin before sarcoidosis was developed, we obtain the following results

1) Persons who, just before contracting sarcoidosis showed a negative tuberculin reaction, or had never had primary tuberculosis, or had never been vaccinated with BCG were also tuberculin negative when they had sarcoidosis. They are, and remain, *primary tuberculin anergic.*

2) Persons who, before contracting sarcoidosis were tuberculin positive after primary tuberculosis or BCG-vaccination, reacted also positively in 60 of the cases, at the first test made while they were suffering from sarcoidosis. However the more prolonged the disease or the more severe its course the weaker this reaction will become during the illness. Since these persons finally become completely tuberculin negative, we call them *secondary tuberculin anergic* cases, because a previously positive tuberculin test has become negative owing to the influence of sarcoidosis.

3) In secondary anergic persons, this phenomenon is, in any case, definit if no healing occurs. In cases where complete cure takes place however the tuberculin test will again become positive with the normalization of all the findings. On th other hand, subjects, who were primary anergic, will remain negative unless a primary tuberculous infection intervenes.

4) Between these two extreme forms of complete primary and complete secondary anergy there are cases with *relative anergy* where the tuberculin reaction is decreased but not entirely suppressed. In Figure 1 these relationships are summarized.

The figure shows the positive reactions obtained *solely by aqueous tuberculin*, and also the positive reactions produced by oily depot tuberculin. Furthermore, cases have been included where we knew that, previously they must have been tuberculin positive — either owing to primary infection or to BCG vaccination — but now react negatively because of the complete suppression of the tuberculin reaction complete secondary anergy. If these cases of proven complete secondary anergy are added to the tuberculin positive cases, the curve of the tuberculin positive subjects is strikingly similar to that of the tuberculin index of the healthy population, which is indicated by a broken line.

What can be said about BCG vaccination and primary or secondary anergy? In sarcoidosis there is mostly no local reaction to BCG-vaccination and even when higher doses are administered it is not possible to obtain a definite positive tuberculin reaction. In spite of this, we believe that a BCG-vaccination, in sarcoidosis with primary tuberculin anergy may be as effective as in normal tuberculin negative persons. This thesis is supported by the two following observations
1) Primary anergetic persons with sarcoidosis who have been inoculated with a strong BCG-vaccination will show a positive tuberculin test after complete cure of sarcoidosis.
2) Since 1950, all our sarcoidosis patients who display an absolute, negative tuberculin reaction are inoculated with a strong BCG-vaccination. There is no danger in vaccinating persons with secondary anergy. Since 1950 we have never observed the development of supervening tuberculosis or of a so-called "Transition into Tuberculosis"

Conclusions

1) A positive tuberculin test in sarcoidosis is due exclusively to primary tuberculosis previously contracted, or to BCG-vaccination.

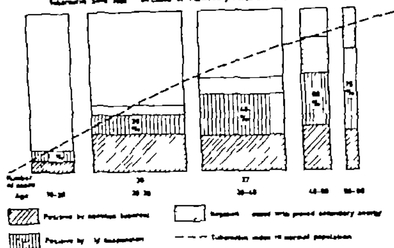


Fig. 1

2) Contrary to tuberculosis, sarcoidosis does not induce real positive tuberculin reaction.

3) A complete negative tuberculin reaction in sarcoidosis is due either to *primary tuberculin anergy* in subjects who have never come in contact with tuberculosis or were never vaccinated with BCG or is the result of complete depression of previous, natural tuberculin allergy which we call *secondary tuberculin anergy*.

4) There are no reasons for regarding the generally observed depression of tuberculin reactivity in sarcoidosis as specific event, and therefore it does not represent any immunologic abnormality in the sense of positive anergy to tuberculosis.

5) BCG-vaccination in sarcoidosis with primary tuberculin anergy may be as effective as in normal tuberculin negative persons.

6) With the progressive decrease of tuberculous infection in the population, the tuberculin test in patients affected with sarcoidosis, particularly in young subjects, will become increasingly negative from the onset of the illness and will remain negative. Consequently the negative tuberculin test in sarcoidosis will be of less diagnostic significance in the future.

From the University of Texas, Dallas, Texas

Cutaneous Reactions of Household Contacts of Sarcoidosis Patients[1]

John S. Chapman W E. Potts, B G Black, Margaret Dyerly and Maro Speight

Knowledge of the delayed type hypersensitivity of the household contacts of patients with sarcoidosis is rather limited. If as Buck and McKusick have maintained (1) there is some tendency for sarcoidosis to occur more frequently in the families of index patients than in the families of matched control index patients, it seemed possible a familial study might be indicated. Since serological tests in adult patients with sarcoidosis revealed a striking prevalence of antibodies against unclassified mycobacteria (2—4) and since Buck and Sartwell (5) showed the prevalence of hypersensitivity to human tuberculin to be no higher among first-degree relatives of patients with sarcoidosis than in control families, it seemed worth-while to investigate delayed hypersensitivity to unclassified mycobacterial antigens in the immediate contacts of sarcoidosis patients.

A preliminary communication (6) reporting results of the first steps in this study has appeared. In this communication it was shown that household contacts of sarcoidosis patients reacted to available antigens of unclassified mycobacteria in the same way as contacts of patients with ca ntary mycobacterial disease and at about twice the rate of controls. The present study involves additional cases and their contacts and includes the results of retesting a number of contacts one and two years following the original skin test. This part of the problem seemed to be worthy of study since it is conceivable that the families of sarcoidosis patients might vary in their reactivity in a different fashion from the variance already shown to exist in mycobacterial contacts (7) Finally it was desired to study the delayed-type hypersensitivity of the patients themselves. While it is clearly established that sarcoidosis reduces all tuberculin-type hypersensitivity (8—11) the possibility might exist that a return of reactivity would permit a response to mycobacterial antigens though response to human tuberculin might be negative.

Materials and Methods

Twenty-one index cases of sarcoidosis (including 16 already reported) and their 63 household contacts were available for study. Fifty-one of the contacts have been retested at yearly intervals once and part of them twice. Eight of the 21 adults have reported for current skin tests.

Materials used for testing have been 1—1000 Old Tuberculin prepared and standardized by the Texas State Department of Public Health. Antigens for unclassified mycobacteria have been prepared in the senior author's laboratory as described elsewhere (2) and standardized to contain a similar amount of biuret nitrogen as that determined in this laboratory for the Old Tuberculin used.

0.1 ml each of 1—1000 dilution of antigens for Group I, Group II and Group III mycobacteria and of 1—1000 Old Tuberculin was injected

[1] This work was supported by Grants E-2065 and AI-04200 National Institutes of Health U.S. Public Health Service.

Table I. Reactions of sarcoidosis contact cases* to group I mycobacterial antigen

Reactions	Number	Per Cent
> 5 mm	27	43
< 5 mm	15	24
Negative	21	33
O.T pos.	1	

Table II. Changes in reactivity of sarcoidosis contacts

Contacts	1—2 years
Increase in diameter	13
Decrease in diameter	13
Significant reactions becoming negative	7
Minor reactions becoming negative	6
No change in diameter	5
Consistently negative	4

Table III. Reactions of sarcoidosis patients

Patient	OT	I	II	III
G	0	20	0	0
L	18	10	0	0
C	0	2 × 10	0	0
D	15	8	10	7

Negative to all 4

Table IV. Skin reactions of M. Kansasii contact cases

Reactions	Number	per cent
> 5 mm	27	42
< 5 mm	19	25
Negative	25	33
Pos. to O.T	1	

intradermally and the reactions measured for induration at 72 hours. All tests have been applied by one of two experienced individuals and read by the same individual. Lately 26 contacts have been tested with an antigen from "wild" soil mycobacterium which has been very reactive with sarcoidosis serum.

Results

Of 63 contacts 27 produced induration greater than 5 mm in diameter to Group I antigens. Reactions to Group II and Group III antigens were generally small in size and were secondary responses. Fifteen additional individuals produced reaction between 1 and 5 mm in diameter and 21 were completely negative. A reaction of 12 mm to Old Tuberculin was encountered in one child who had had known undependent contact with tuberculosis. All other children were completely negative to Old Tuberculin on one, two, or three tests.

Of those who were retested at one or two year intervals it was found that in the period involved 13 had shown decrease in response, 13 an increase, and 9 had no change. Of those whose reactions diminished, 7 had had significant reactions and 6 had had less than 5 mm induration on the first test. Five contacts who changed from definitely positive reactions to negative or insignificant reactions were in the same family.

Of the index cases tested with these antigens 4 patients were negative to both mycobacterial and tuberculosis antigens. 1 had a 20 mm reaction to Group I antigen and her only child had a similar reaction. Another patient had a curious 2 × 10 induration to Group I antigen.

Only 1 of this patient's 6 contacts showed response to any antigen. Two patients had responses of 15 mm and 18 mm to human tuberculin, with weaker responses to unclassified antigens. The only child of the 15 mm reactor at one time produced induration to Group I antigen, but this response became negative in the course of 1 year. The other patient who reacted to tuberculin has 6 children, all of whom are negative to human antigen. Three of these reacted to Group I antigen and 3 (including 1 child born in the period of observation) are negative to all antigens.

Of the 26 sarcoidosis contacts tested with antigen derived from T6 D6, a soil scotochromogen, 5 individuals produced induration, none of which exceeded 4 mm in diameter

Discussion

It will have been noted that in our particular area nearly all significant and all leading reactions have been to Group I antigen, while our Group II and Group III antigens have elicited very few reactions. This point is the more remarkable that sarcoidosis patients in this area have significant and frequently leading serological reactions to Group II and Group III antigens. The number of patients who have been tested at the same time both serologically and intradermally is as yet too small to allow more extensive comment.

In the previous paper it was shown that contacts of sarcoidosis and contacts of cavitary mycobacterial disease react to the various unclassified antigens in a very similar fashion, the figures being 34 and 36 respectively 43 % of the present group of sarcoidosis contacts, counting the sole or the last test, produced induration of 6 mm or more to Group I antigen. For purposes of present comparison a similar group of contacts of M kansasii patients has been studied In this hitherto unreported group there were 76 contacts of 28 patients, 32 or 42 1 % of these contacts had indurations of 6 mm or larger while 25 or 33 were entirely non-reactive. Of those who had a second test one year later 8 had increased reaction to a significant diameter while 6 previously clearly positive had developed insignificant reactions. The major difference between the two groups lies in the fact that the M. kansasii contacts were a bit older 48 under 10 years of age as compared with 62 of the sarcoidosis contacts.

Lack of significant reaction to the antigen of T6 D6 is only to be expected in that antigens of other scotochromogenic organisms employed in humans known to harbor such organisms have failed to elicit significant response. The implication seems to be that though Group II organisms may elicit circulating antibodies their capacity to produce significant or persistent tuberculin type allergy is quite limited.

Antigens of organisms of the unclassified mycobacterial groups present curious contradictions between their capacity to elicit skin reactions and to produce circulating antibodies. The PPD-B of the U.S. Public Health Service for example, elicits a high percentage of skin responses, yet in our laboratory it is very rare to encounter antibodies in sera to P-39 antigen. Conversely while many serological reactions to antigens of P 2 and P 17 are demonstrable, it is very rare to encounter skin reactions of significance to these same antigens. Yet P 39 the source organism for PPD-B, and P-2 and P 17 are members of Runyon's Group III This difference is also prominent in the serological reactions of a group of children studied by Dr David T Smith. Though many reacted to avian tuberculin, few showed antibodies to avian antigens in agar diffusion.

This study bears out the findings of the preliminary report in showing that contacts of sarcoidosis manifest a capacity for delayed type hypersensitivity that they do not show an unusual prevalence of reactions to Old Tuberculin, that they react to unclassified mycobacterial antigens in essentially the same manner as contacts of M. kansasii patients, which is more than twice the rate for 500 controls studied by Dewlett et al. (12) This difference is significant ($P < .01$) This study further indicates a fluctuation in the level of tuberculin-type response, common to both groups of contacts and at about the same frequency The variance in this type of sensitivity to M. kansasii antigen has been noted previously (7)

Conclusions

1 Household contacts of patients with sarcoidosis react to antigens of unclassified mycobacteria and to human tuberculin with the same frequency as contacts of M kansasii patients.

2. The rate for reaction to unclassified mycobacterial antigens in both groups is more than double that of controls and the difference is statistically significant.
3. Fluctuations in the level of tuberculin-type allergy take place to about the same extent and with about the same frequency in both groups.
4. I [illegible] ery small group tuberculin-type allergy to Group I unclassified mycobacterial antigen has been demonstrated in 2 sarcoidosis patients who showed no response to human tuberculin.
5. Tuberculin-type allergy or lack of it in contacts is independent of skin reactions in the index cases.

References

1. Buck, A. A., and McKusick, V. A. Epidemiological investigations of sarcoidosis. III Serum proteins syphilis association with tuberculosis familial aggregation. Amer J Hyg. 74 174 1961
2. Chapman, J S A serological reaction associated with sarcoidosis. Proc. Soc. Exper Biol. and Med. 107 321 1961
3. Chapman, J. S. Mycobacterial and mycotic antibodies in sera of patients with sarcoidosis. Ann. Int. Med. 55 918, 1961
4. Chapman, J. S and Speight, M. This journal.
5. Buck, A. A., and Sartwell, P E. Epidemiological investigations of sarcoidosis. II Skin sensitivity and environmental factors. Amer J Hyg. 74 172, 1961
6. Chapman, J S., Potts, W E., Speight, M., and Dyerly M., Cutaneous reactions of household contacts of patients with established diagnoses of sarcoidosis. Amer Rev Resp. Dis. 88 95, 1963.
7. Chapman, J S., Dewlett, H. J and Potts, W E. Cutaneous reactions to unclassified mycobacterial antigens A study of children in household contact with patients who excrete unclassified mycobacteria. Amer Rev Resp. Dis. 86: 547 1962.
8. Buck, A. A., and Sartwell, P E. op. cit.
9. Sones, M., and Israel, H. L. Altered immunologic reactions in sarcoidosis. Ann. Int. Med. 40: 260, 1954.
10. Hoyle, C., Dawson, J and Mather H. Skin sensitivity in sarcoidosis. Lancet 2: 164, 1954
11. Citron, K. M. Skin tests in sarcoidosis. Tubercle 38: 33, 1957
12. Dewlett, J J., Leflowitz, L., Fulbyfil, R., Christianson, L. C., and Chapman, J. S. A study of reactions to various anonymous mycobacterial antigens in Dallas County orphans. Paper presented at annual meeting of American Trudeau Society Los Angeles, Calif May 1960.

Dr Hosoda It is accepted that tuberculin sensitivity in sarcoidosis is very often attenuated, but the rate of the lowering in sensitivity varies with reporters. The authors observed chronologically 15 cases with sarcoidosis, comprised of 7 with a histologic evidence and 8 with a clinical compatibility After the onset of the disease, most of them were repeatedly tuberculin-tested. The results revealed that the negative tuberculin rates varied from 13.3 % to 73.3 depending on the stage of the disease. Generally it seems that most of the cases have a period when tuberculin sensitivity is very lowered. In this study many cases regained tuberculin sensitivity as the chest findings were improved. The grade of regained tuberculin sensitivity was usually as high as that before the onset of the disease. However it should be noticeable that there were a few cases which kept a high sensitivity to tuberculin throughout the period of pulmonary manifestation, though repeatedly tuberculin-tested.

In addition to this observation, 14 cases with primary hilar adenopathy diseases such as Hodgkin disease were tuberculin-tested. These cases showed a similar lowering of tuberculin sensitivity to the cases with sarcoidosis.

Dr Reid Very good evidence has been produced by Lowe and McNulty (*Leprosy Review* *4* 61 1953) that the delayed type of hypersensitivity reaction of tuberculin is depressed in leprosy Their actual figures were — 75.2 of tuberculin positive in 359 healthy subjects, but only 55—59 in tuberculous and lepromatous leprosy patients, of whom there were 356.

I am aware that Dr James has figures which do not show this, but the work reported by Lowe and McNulty appears good and the results significant I wonder therefore if depression of delayed or Mantoux type reactivity is not a general phenomenon which can be seen in variety of cases and whether anyone can inform us if the reactivity to say candidin or to atypical mycobacterial tuberculins is less in cases of tuberculosis than in the general healthy population.

Dr Kooij This depression of the tuberculin reaction in leprosy as reported by Lowe and McNulty could be confirmed by Kooij and Rutgers in their article on Leprosy and Tuberculosis. A comparative study with the aid of skin tests with tuberculin, killed BCG and the Dharmendra lepromin in South African Bantus (Internat. Journ. of Leprosy vol. 26, p. 24—40, 1958) They found a striking number of patients with leprosy who showed no reaction to tuberculin and BCG although in many of them by chest X-rays calcified foci in the lungs were detected. This suppression of the tuberculin reaction, especially in tuberculoid leprosy is in support of the view that tuberculoid leprosy belongs to the syndrome sarcoidosis.

Dr Scadding Some of our observations, which I was not able to mention in my paper for lack of time, may be relevant to Dr Sommer results. We analysed the tuberculin sensitivity of patients with sarcoidosis according to whether they did or did not have independent evidence of a previous tuberculous infection, in the form of an undoubted past history or calcified residues of primary infection[1,2]. There was no difference in the level of tuberculin sensitivity in the two groups. This observation is more in favour of the view that the depression of tuberculin and similar delayed skin sensitivity in sarcoidosis is part of an immunological state which is a pre-requisite for the development of sarcoidosis, rather than of the alternative view that some unidentified agent causing sarcoidosis also depresses tuberculin sensitivity. With regard to Dr Reid s question about tuberculin sensitivity in tuberculosis, it is of course well recognised that rarely patients with indolent forms of pulmonary tuberculous may show persistent non-reactivity of the skin[3,4]. The clinical and radiographic picture in some of these patients so closely resembled that of sarcoidosis that, apart from the finding of tubercle bacilli, which is to some extent a matter of chance, they might have been categorised as sarcoidosis.

1 Citron K. M. and Scadding, J G (1957) Quart J Med. 26 277

2 Scadding J G (1961) Tubercle Lond. 42 121

3. Mascher W (1951) Amer Rev Tuberc 56. 198

4 Scadding J G (1956) Tubercle Lond. 37 371

OCULAR SARCOIDOSIS

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The Diagnosis and Treatment of Ocular Sarcoidosis

D Geraint James

Whereas sarcoidosis is only responsible for about 4 per cent of cases of uveitis, eye involvement, predominantly uveitis, occurs in about one-quarter of patients with sarcoidosis. Although the ocular lesion is but an incident in generalized disease, it is the most troublesome and incapacitating. Its early recognition and treatment may prevent both protracted suffering and blindness. We have observed ocular involvement in 123 of 442 (27.8 per cent) patients with histologically-confirmed generalized sarcoidosis. Patients with clinical, radiological and immunological features of sarcoidosis have been excluded from this analysis if there was no histological confirmation.

Ocular lesions They comprised	patients
Anterior Uveitis	89
Posterior Uveitis	12
Corneo-sclero-conjunctival involvement	32

Anterior uveitis. This is by far the commonest mode of presentation of ocular sarcoidosis. It consisted of 34 patients with acute iridocyclitis and 55 with chronic iridocyclitis the differences between the acute and chronic disease being striking (Table I). Acute iridocyclitis has an abrupt onset usually in the third decade of life with evidence of ciliary congestion, and turbid aqueous and keratic precipitates. By contrast chronic iridocyclitis presents insidiously in an older age group. There is no evidence of acute inflammation in the anterior chamber but instead there are fatty granulomatous nodules attached to the iris and post-corneal surface, synechiae sticking iris to lens, and lens opacities. The differences are as clear cut as those between acute and chronic hepatitis or between Types I and II nephritis. Whereas there are virtually no complications of acute iridocyclitis, chronic uveitis progresses relentlessly towards further fibrosis with the production of secondary glaucoma, cataract formation and ultimate blindness. The differences extend to tissues other than the eye. Acute iridocyclitis is associated with transient intrathoracic lesions, and transient skin lesions (commonly erythema nodosum) whereas chronic iridocyclitis is associated with pulmonary fibrosis, chronic persistent skin lesions (commonly lupus pernio) and with bone cysts which constitute the hallmark of chronicity of sarcoidosis.

Posterior Uveitis is difficult to visualise in the presence of anterior uveitis. It is undoubtedly commoner than is realised. Focal choroidal nodules appearing as peripheral exudates sometimes causing venous constriction and periphlebitis, or retinal oedema, or nonspecific periphlebitis retinae were observed in 12 patients. It was overshadowed as a presenting feature of sarcoidosis by accompanying anterior uveitis.

Corneo-sclero-conjunctival involvement consisted of

	patients
Conjunctivitis	14
Phlyctenular	6
Nonspecific	8
Keratoconjunctivitis sicca	10

TABLE I A comparison of acute and chronic uveitis in 89 patients with anterior uveitis due to sarcoidosis

		Acute	Chronic
Number of patients		34	55
Bilateral		24	52
Onset		Sudden	Insidious
Decade of onset (years)		20—30	40—50
Signs		Ciliary congestion turbid aqueous K.P	Fatty nodules synechia iris → lens
Sequelae		None	Lens opacities Glaucoma Cataract Blindness
Chest X-ray	resolution	29/29 (100 %)	7/34 (20 %)
Skin lesions	Erythema nodosum	12	3
	Other	2	24
Bone cysts		0	9
Spleen		3 (9 %)	11 (20 %)
Bell Palsy		4 (11 %)	2 (4 %)
Lacrimal gland involvement		0	1
Parotid gland involvement		1	7
Duration before Cortison		Nil	1—14 years

K. P Kerati precipitates.

Conjunctival follicles	5
Scleral plaques	3

There are no clearcut features by means of which *sarcoid conjunctivitis* can be segregated. *Phlyctenular conjunctivitis* presenting in adult life is more likely to be due to sarcoidosis than to tuberculosis. If there are associated conjunctival follicles, then they should be biopsied for histological evidence of sarcoidosis. The most distressing type of conjunctival involvement is *Keratoconjunctivitis sicca* in which dry eyes are associated with corneal degeneration and staining

Ocular Syndromes

There are certain well-defined clinical syndromes of sarcoidosis with eye involvement (table II)

Acute iritis erythema nodosum and bilateral hilar lymphadenopathy has a benign self limiting course. The prognosis is excellent for complete resolution within one year is the rule.

Chronic iridocyclitis lupus pernio, bone cysts and pulmonary fibrosis. Irreversible fibrosis has occurred in all involved tissues, so ultimate resolution is unlikely to occur Systemic corticosteroids provide symptomatic relief but do not lead to resolution of the pathological process.

Keratoconjunctivitis sicca with or without parotid and lacrimal gland involvement mimics Sjögren's syndrome, with its distressing symptoms of dry eyes and dry mouth, but there is no joint involvement. Oral corticosteroids may be needed to reinforce topical applications. Eventual resolution can be anticipated, but it may persist for several years.

Bell's palsy with anterior uveitis and parotid gland enlargement conveys features of Heerfordt's syndrome It is worth treating such cases with early and vigorous oral corticosteroids.

TABLE II.

Ocular syndromes	Number of patients
Acute iritis	
Erythema nodosum	12
Bilateral hilar lymphadenopathy	
Chronic iridocyclitis	
Lupus pernio	9
Bone cysts	
Pulmonary fibrosis	
Keratoconjunctivitis sicca	
(With parotid and lacrimal gland	10
enlargements in 5 patients)	
Bell's palsy	
Parotitis	6
Anterior uveitis	

Relationship of Ocular to Generalised Sarcoidosis

Age and sex incidence. Acute iridocyclitis predominated in the third decade and chronic iridocyclitis in the fifth decade and this difference probably accounts for the fairly even overall distribution of all types of eye lesions in the third, fourth and fifth decades. The age range is similar to that observed with skin lesions; both are older groups than those without eye lesions where the peak incidence of onset is in the twenty to thirty decade.

Sarcoidosis affects both sexes to about the same extent but in those patients with ocular lesions (and again like those with skin lesions) there was a preponderance of females (83 of 123 patients).

Intrathoracic involvement. This occurred in 91 of 123 (74 per cent) patients with ocular disease, the changes ranging from the early stage of bilateral hilar lymphadenopathy to the oldest stage of diffuse pulmonary mottling without hilar adenopathy (Tables III). Those with long-standing ocular disease had intrathoracic changes at an older stage of development. The chronicity of the eye lesions was matched by irreversibility of lung lesions. Thus, intrathoracic abnormalities resolved in all patients with acute iridocyclitis and acute conjunctivitis, but in only 7 of 34 (21 per cent) patients with chronic iridocyclitis. Complete radiological clearing was achieved in 56 of 91 (61 per cent) patients with all types of ocular sarcoidosis.

Skin lesions. These included lupus pernio, maculopapular eruptions, persistent plaques, scars or erythema nodosum. They occurred in 66 of the 123 (54 per cent) eye patients, comprising 30 with erythema nodosum and 33 with various other more chronic skin lesions. The simultaneous occurrence of skin plaques or erythema nodosum with uveitis should always arouse the suspicion of sarcoidosis.

Lymphadenopathy. Enlarged palpable lymph nodes were present at some stage of the disease in 38 per cent of patients with ocular disease compared with 31 per cent of the group without eye manifestations or 33 per cent for the whole series.

Splenomegaly. A spleen was palpable in 22 of 123 (18 per cent) with eye lesions compared with 9 per cent without ocular disease or 12 per cent for the whole series.

Bone cysts. Cystic changes in bones of the hands or feet were found radiologically in 11 patients, 3 of whom had chronic anterior uveitis. All had accompanying skin lesions.

Serum globulin levels. Raised or abnormal serum globulin levels were noted in 30 of 80 (38 per cent) patients in whom they were determined. There was no significant difference whether ocular lesions were present or absent.

Serum and urine calcium levels. Hypercalcaemia was found in 9 of 72 (12 per cent) with ocular disease the same incidence as 30 of 256 (12 per cent) in the whole series. Hypercalciuria, as judged by repeated 24-hour urine determinations, was noted in 5 of 18 (28 per cent) ophthalmic patients and 19 of 65 (29 per cent) of all patients.

Skin Tests

Mantoux Reaction. Sixty-eight of 108 (63 per cent) with ocular involvement gave negative results with 100 tuberculin units, compared with 61 per cent with skin lesions or 54 per cent for the whole series.

Kveim tests. These provided histological confirmation in 77 of 95 (81 per cent) oph-

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Bell's palsy with anterior uveitis and parotid gland enlargement convey features of Heerfordt's syndrome. It is worth treating such cases with early and vigorous oral corticosteroids.

Dr Brun J'ai beaucoup étudié les tuberculoses oculaires. Celles-ci existent de façon formelle et il ne serait être question de dire qu'elles doivent être maintenant assimilées à une étiologie sarcoïdosique.

En dehors du fait qu'elles peuvent survenir sur un terrain récemment et manifestement primo-infecté par le bacille de Koch (conjonctivite phlycténulaire) par exemple, ou lors de disséminations hématogènes post-primaires (iritis, kératite) il y a des cas pour lesquels les atteintes oculaires accompagnent des adénopathies bacillaires périphériques, prouvées bactériologiquement et histologiquement et précédant en même temps que ces adénopathies. Ces atteintes oculaires, nous les avons appelées paraganglionnaires elles coïncident dans certains cas avec des atteintes rhumatismales et cutanées.

Dr James Having heard so many of you discuss tuberculosis of the uveal tract, I feel I must put on record that it is extremely rare, if indeed it still exists. Perhaps the commonest way in which tuberculosis affects the eye is in the form of choroidal tubercles in tuberculous meningitis, but this is extremely rare other than as part of uveitis.

Ophthalmologists apply cortisone to the conjunctiva and even by injection into the eye in acute inflammation of the uveal tract without being apprehensive of the possibility of flaring up tuberculosis of the eye.

Dr Siltzbach *When Dr James says that he does not believe that tuberculosis of the eye exists,* he is taking poetic licence. He may be allowed to do this because he is Welsh and all the Welsh are poets. Despite his exaggeration, he is making an important point. In the United States the whole approach to uveitis changed when it was found that the anti-tuberculosis drugs, so effective elsewhere in the body, were almost wholly ineffective in uveitis. Then the frequency of ocular sarcoidosis became common knowledge and corticosteroid therapy locally and systemically took over. This could hardly have been possible if the ophthalmologist retained his original concept that most uveitis was the result of an infection with the tubercle bacillus.

Dr Nosaki As shown in the accompanying table, among our 183 samples conforming with the categories I, II and III, according to the classification by the medical group on the Washington Conference, ocular manifestations were seen in 61 cases (32.9 %).

Table I. Sites of lesions of sarcoidosis[a]

Groups	I—III		IV	
	183		97	
Total	Cases	100.0	Cases	100.0
Site				
Lung	104	56.2	53	54.6
BHL	145	78.4	96	99.0
UHL	5	2.7	1	1.0
Other Lymph Nodes	52	28.1	5	5.2
Skin	56	30.3	19	19.6
Eye	61	32.9	16	16.5
Bone	10	5.4	1	1.0
Parotid	8	4.3	4	4.1
Nerves	8	4.3	5	5.2
Other Organs	18	9.7	4	4.1
Total	183	100.0	97	100.0

The systematized samples chosen out of 454 cases collected through the nation-wide survey by the Sarcoidosis Research Committee in Japan.

The groups according to the classification by the Medical Group on the II. International Conference on Sarcoidosis, 1960.

Among those 56, there was one case of erythema nodosum.

Dr Siltzbach The percentage of ocular involvement with sarcoidosis in any given series depends, in great degree, upon the manner in which the patients with sarcoidosis are collected. Obviously if most of the patients are referred from ophthalmologists, the proportion of patients with ocular sarcoidosis rises greatly. The same is true of skin sarcoidosis, bone sarcoidosis, etc. The percentage of involvement of any organ in sarcoidosis can only be given its full meaning when one knows approximately in what stage of the illness the specified organ involvement occurs. By that I mean, a series which is composed almost wholly of asymptomatic patients referred through mass survey units will contain fewer patients with extrathoracic manifestations than a series of patients collected at a hospital where symptomatic patients are being cared for. I would be interesting to learn from Dr Löfgren the proportion of his patients with the early hilar node syndrome who already show ocular sarcoidosis.

Table III Intrathoracic radiological changes in 123 patients with ocular sarcoidosis

Type of ocular lesion	No. of patients	Chest X ray stage[1]				Subsequent complete clearing	
		0	1	2	3	No.	
Iridocyclitis	89	26	30	19	14	36	57
Acute	34	5	18	9	2	29	100
Chronic	55	21	12	10	12	7	20
Corneo-sclero-conjunctival involvement	32	6	18	4	4	19	73
Cataract	1	0	0	1	0	0	
Retinitis proliferans	1	0	0	1	0	1	
Total	123	32	48	25	18	56	61

Stage 0 Clear Chest Radiograph. Stage 1 Bilateral hilar lymphadenopathy Stage 2 Hilar lymphadenopathy and Pulmonary mottling Stage 3 Diffuse Pulmonary mottling

thalmic patients, or 329 of 380 (86 per cent) in the whole series of patients with histological proof.

Diagnostic Routine

1) Slit lamp examination of both eyes and ophthalmoscopy since asymptomatic ocular lesions may only be revealed by these measures.
2) Chest radiography since the chest X-ray is abnormal in three-quarters of patients with ocular sarcoidosis.
3) Serum calcium level, since it is elevated in 12 per cent of sarcoidosis patients, both with and without ocular involvement.
4) Histological confirmation by
   (a) Biopsy of conjunctival follicles, skin or lymph node when these tissues are obviously involved.
   (b) If there is no evidence of clinical involvement, blind biopsy of the right scalene node if there is hilar adenopathy or otherwise aspiration liver biopsy
   (c) Kveim test.

Treatment

There is only one form of treatment which is at present known to influence clinical radiological and histological features of sarcoidosis—namely corticosteroid therapy Its main indication is ocular sarcoidosis. There is no indication for giving anti-tuberculous chemotherapy or any other antibiotics with it.

Corticosteroids may be given topically in the form of eye-drops, perhaps with subconjunctival cortisone, in the first instance. Oral corticosteroid therapy is given if there is no rapid and favourable response to topical administration, or if posterior uveitis is detected. Prolonged and energetic oral corticosteroid therapy is necessary for posterior uveitis.

Summary

Ocular lesions were observed in 123 of 442 (27.8 per cent) patients personally observed with histologically-confirmed generalised sarcoidosis. They included acute and chronic uveitis, conjunctivitis, keratoconjunctivitis sicca and conjunctival follicles. There were striking differences between acute and chronic anterior uveitis (Table I). Certain well-defined clinical syndromes were delineated (Table II). The intrathoracic radiological changes are noted in ocular sarcoidosis (Table III) and compared with other types of sarcoidosis. Ocular lesions are but an incident in a generalised disease involving several other tissues and systems. A diagnostic routine is laid out, and the management of ocular sarcoidosis with corticosteroids is outlined.

Aus dem Institut für Humangenetik, Göttingen, Bundesrepublik Deutschland

Die Genetik der Sarkoidose

Gerhard Jörgensen

Die Frage nach genetischen Einflüssen bei der Sarkoidose ist bisher nur aufgrund kasuistischer Mitteilungen diskutiert worden. — Ich habe deshalb in der Zeit vom 1. 4. 1960 bis 30. 9. 1961 über die Gesundheitsämter der Bundesrepublik Deutschland und West-Berlins 2471 Sarkoidose-Patienten ermittelt, um auslesefreie Zwillings- und Familienuntersuchungen durchzuführen.

Unter diesen 2471 Patienten sind 15 Zwillinge, deren Partner noch leben (Tab. I). Von den 4 eineiigen Zwillingen sind 2 bezüglich der Sarkoidose konkordant, 2 diskordant. Von den 11 zweieiigen Paaren ist nur ein PZ-Paar konkordant, die 10 anderen sind diskordant.

Bei 40 von 2471 Sarkoidose-Patienten kommt die gleiche Erkrankung auch bei Blutsverwandten vor, und zwar sind in 21 Fällen nur Geschwister und in 18 Fällen ein Elter betroffen (Tab. II). In einem Falle sind *4 von 7 Geschwistern*, beide Eltern, die Schwester des Vaters sowie deren Tochter an Sarkoidose erkrankt (Abb. 1). Der Vergleich des klinischen Bildes von Familienangehörigen zeigt, daß die Lokalisation des Prozesses vor allem im Beginn, aber auch im späteren Verlauf innerhalb der Familie überwiegend ähnlich oder gleich ist. Andererseits spricht die gelegentlich beobachtete Vielfalt der Organmanifestation innerhalb einer Familie dafür, daß es sich bei der Sarkoidose um ein einheitliches Krankheitsbild handelt, was gelegentlich noch angezweifelt wird.

Von Interesse ist, daß auch in der therapeutischen Ansprechbarkeit sowie Nichtansprechbarkeit auf ACTH- und Cortison Übereinstimmungen bei Geschwistern vorzuliegen scheinen.

Wenn man die Literaturkasuistik und die Ergebnisse der eigenen Zwillings- und Familienuntersuchungen überblickt, so bestehen keine Zweifel, daß am Zustandekommen der Sarkoidose, an der Lokalisation und dem Verlauf genetische Einflüsse beteiligt sind. Wenn Erbanlagen keine oder nur geringe Bedeutung für die Erkrankung hätten, und nur eine Infektion ausschlaggebend wäre, müßten Ehepartner infolge des engen Zusammenlebens ebenfalls öfter gemeinsam betroffen sein, was jedoch nicht der Fall ist. Meine Nachforschungen darüber, ob ein Sarkoidose-Kranker evtl. einen Mitmenschen infiziert haben könnte, blieben bis auf drei Fälle, in denen die Möglichkeit, nicht jedoch die Wahrscheinlichkeit einer Kontaktinfektion gegeben ist, ergebnislos.

Welcher Art sind die genetischen Faktoren, die der Reaktionsbereitschaft des Organismus, an einer Sarkoidose zu erkranken, zugrunde liegen?

Ein klassischer „mendelistischer" Erbgang, dominant oder rezessiv, liegt nicht vor und war von vornherein nicht zu erwarten.

Dr *Lofgren*: I fully agree with Dr James regarding the rarity of tuberculous uveitis. From old text-books of ophthalmology available when I was a medical student, I learnt that tuberculosis was an important cause of chronic uveitis. However, nothing was mentioned, in this respect, about Boeck's sarcoid, or Morbus Schaumann, or lymphogranulomatosis benigna. For many years I have had experience of a clientele of pulmonary tuberculosis, but, personally, I have never seen a case of tuberculous uveitis. Instead, I have seen rather many patients with sarcoidotic uveitis. Consequently, my experience causes me to wonder whether the tuberculous uveitis mentioned in the literature, was not actually of sarcoidotic origin. I have consulted Swedish ophthalmologists about this, and they have shared my opinion.

Dr Siltzbach asked me about the incidence of uveitis in cases of bilateral hilar adenopathy. In the material published by me in 1953, uveitis was found to develop in 13 out of 212 of these cases (6) with roughly the same incidence in cases with and without erythema nodosum. Eight of the thirteen patients had concurrent parotitis. In six cases the uveitis was the first symptom to appear; in five it occurred within 1 to 2 months, and in one case 4 months after the condition was detected.

My impression is, however, that the frequency of sarcoidotic uveitis has decreased during the last ten years. Is that a general experience?

Tabelle III. Probandenmethode — Mittelwerte der Alterskorrektur nach Weinberg und Ilse-Schulz

Zahl der Probanden	Erkrankte Eltern		Korrigierte Bezugszahl	Erkrankte Geschwister			Korrigierte Bezugszahl
	Beide Eltern Sarkoidose	Ein Elternteil Sarkoidose		Zahl der Befallenen Gesch. wenn beide Eltern befallen	Zahl der Befallenen Geschwister ein Elternteil befallen	Zahl der Befallenen Geschwister beide Eltern gesund	
	2	17		3	4	24	
2421		19	4000		31		3,23
	0,47	± 0,108			0,88	0,157	

Tabelle IV. Mittlere Größe und mittleres Gewicht bei männlichen Sarkoidose-Kranken

Zu Beginn der Erkrankung

Altersgruppe	Anzahl	M-Größe (cm)	M-Gewicht (kg)
20—24	51	174,9 + 7,60	70,2 — 8,03
25—29	65	173,1 6,40	71,9 + 9,17
30—34	45	173,6 ± 5,60	74 7 ± 10,86
35—39	27	173,0 ± 6,94	74,6 _ 11,35
40—44	28	172,5 + 5,61	74,3 11,63
45—49	16	170 7 ± 5,78	70,9 7,24
50—54	26	169 9 ± 6,99	70,7 + 14,0
55—59	4	168,0 + 6,45	70,1 ± 5,77

Tabelle V. Mittlere Größe und mittleres Gewicht bei weiblichen Sarkoidose-Kranken

Zu Beginn der Erkrankung

Altersgruppe	Anzahl	M-Größe (cm)	M-Gewicht (kg)
20—24	55	163,9 + 7,88	63,6 + 15,13
25—29	74	164,8 + 5,98	66,0 ± 11,66
30—34	40	162,3 ± 5,84	65,1 + 10 10
35—39	50	161,8 ± 6,49	65,6 ± 8,49
40—44	50	161,8 6,17	65,1 + 9,91
45—49	36	159 4 6,82	62,0 11,66
50—54	40	160,8 + 7 13	66,9 + 11,39
55—59	26	159,6 + 5 45	67 7 11 14
60—64	14	159 4 — 5,73	60,5 + 7 73
65—69	4	159,7 + 3,42	72,9 ± 12,2
70—74	2	161,5	71,5

der durchschnittlichen Bevölkerung. Sie beträgt für Eltern und Geschwister das 20—30-fache.

3. das größenordnungsmäßig nicht signifikant erscheinende Erkrankungswahrscheinlichkeit von Eltern und Geschwistern (wenn man erschiedene umweltbedingte Faktoren in Betracht zieht) (Tab. III)

Die Erkrankungswahrscheinlichkeit beträgt für Geschwister eines Sarkoidose-Kranken 0,88 ± 0,157 %, für Eltern 0,47 ± 0,108 %.

4. die vermutlich erhöhte Erkrankungswahrscheinlichkeit von Kindern, wenn beide Eltern betroffen sind gegenüber Kindern, deren einer Elter betroffen ist.

Die gelegentlich geäußerte Ansicht, daß ein besonderer Habitus, in erster Linie fettleibige Personen für die Sarkoidose-Erkrankung disponiert sind, hat sich nicht bestätigt. Die Berechnung des Verhältnisses von Körperhöhe zu Körpergewicht, getrennt für Ge

Tabelle I Sarkoidose bei Zwillingspaaren

Paare			konkordant	diskordant
EZ	♂ 1 ♀ 3	= 4	2	2
ZZ	♂ 1 ♀ 6	= 7	—	7
PZ		4	1	3

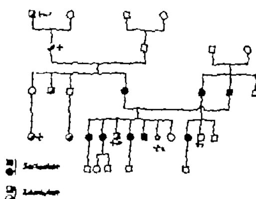


Fig 1

Tabelle II Sarkoidose bei Blutsverwandten

Verwandschaftsverhältnis	Zahl der Beobachtungen
Geschwister	
Bruder/Bruder	7
Bruder/Schwester	5
Schwester/Schwester	7
1 Bruder/2 Schwestern	1
3 Schwestern	1
Eltern/Kind	
Vater/Sohn	1
Vater/Tochter	3
Mutter/Sohn	4
Mutter/Tochter	5
Vater/2 Söhne	1
Vater/2 Söhne/1 Tochter	1
Vater/Mutter 1 Sohn/3 Tochter/Schwester des Vaters/ Tochter der Schwester des Vaters	1
Mutter/2 Söhne	1
Entfernter Blutsverwandte	
Großmutter mütterl./Enkel	1
Vetter/Vetter	1
Bruder/Bruder / Vetter/Vetter[1]	1
Vater/Mutter/1 Sohn/3 Tochter/Schwester des Vaters/ Tochter der Schwester[1]	1

[1] doppelt aufgeführt

Etwa von den Elter/Kind-Kombinationen auf einen dominanten Erbgang oder bei den Geschwisterbeobachtungen auf einen rezessiven Erbgang schließen zu wollen, wäre selbstverständlich falsch. Ich erwähne diese mögliche Fehldeutung jedoch besonders deshalb, weil die klassischen monogenen Erbgänge ganz allgemein auch dort immer wieder diskutiert und mit allerlei hypothetischen ad hoc — Vorstellungen mühsam konstruiert werden, wo aus dem Vorliegen von Zwillings- und Familienkasuistiken, die zudem vielfach einer Interessantheitsauslese ihre Publikation verdanken, lediglich die Feststellung erlaubt ist, daß *genetische Einflüsse überhaupt wirksam sind*.

Zwar ist die Sarkoidose vermutlich eine Infektionskrankheit, jedoch nahe der Grenze, jenseits der die spezifische Natur von Infektionserregern kaum noch Bedeutung hat.

An der Bereitschaft des Organismus, mit einer Sarkoidose zu reagieren, ist offenbar ein polygenes multifaktorielles genetisches System mit breitem Schwellenwerteffekt beteiligt. Dafür sprechen

1. das Konkordanz/Diskordanz-Verhältnis bei eineiigen und zweieiigen Zwillingen (wenn man es trotz der geringen Zahl als real ansieht). Die Konkordanzziffer liegt nämlich bei den EZ um das Zehnfache höher als bei den ZZ.

2. die beträchtlich erhöhte Erkrankungswahrscheinlichkeit naher Blutsverwandter von Sarkoidosekranken gegenüber der in

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Die Blutgruppen bei der Sarkoidose

Gerhard Jörgensen und Karl Wurm

Schon bald nach der Entdeckung des ABO-Blutgruppensystems hat man seine Beziehungen zu bestimmten inneren Krankheiten untersucht (Literatur bei Hirszfeld 1928, Steffan 1930, 1932). Nichtgeeignetes Untersuchungsgut und unzureichende Methodik wurden den Untersuchungen jedoch zum Verhängnis. Erst mit der Arbeit von Aird, Bentall und Fraser Roberts (1953), in der die Korrelation zwischen der Blutgruppe A und der Häufigkeit des Magenkrebses mit moderner statistischer Methode nachgewiesen worden ist, hat die Forschung über die natürlichen selektiven Wirkungen im ABO-Blutgruppensystem neuen Auftrieb und ein stabileres Fundament bekommen.

Die Korrelationen zwischen Blutgruppen und Infektionskrankheiten hat besonders Vogel mit seinen Mitarbeitern Helmbold und Pettenkofer (1960, 1961) untersucht. Die Autoren weisen auf ein von den ABO-Blutgruppen abhängiges Verhalten der Wassermann-Reaktion nach antiluischer Therapie hin. Vor allem diskutieren sie jedoch die wahrscheinliche Bedeutung des ABO-Systems im Rahmen der selektiven Wirkungen der großen Seuchen wie Pocken und Pest.

Nach Vogel und Mitarbeitern verlaufen die Pocken bei Patienten der Blutgruppe A und AB schwerer als bei denen mit den Gruppen B und 0. Vaccinations-Encephalitiden kommen dementsprechend bei Trägern der Gruppe A und AB häufiger vor. Ursache ist vermutlich ein A-Antigen oder eine Substanz mit entsprechender Antigeneigenschaft des Pockenvirus.

Aus der geographischen Verbreitung der Blutgruppe 0 mit relativer Seltenheit in den alten Pestzentren leiten Vogel und Mitarbeiter den vermutlichen Selektionsnachteil der Gruppe 0 gegenüber der Pest ab. Der Gehalt von H-Antigenen in Pestbazillen, die einen Nachteil für die Blutgruppe 0 bedeuten, spricht in diesem Sinne.

Angeregt durch diese Arbeiten haben wir Blutgruppenuntersuchungen bei Patienten mit Sarkoidose durchgeführt.

Der Vergleich unserer Ergebnisse an 518 Sarkoidose-Patienten mit der Verteilung der ABO-Blutgruppen in Deutschland ergibt eine um 4,81 größere Häufigkeit der Blutgruppe A. Die A-Eigenschaft nimmt dabei vor allem auf Kosten der Blutgruppe B (— 2,7) weniger der Gruppe 0 (— 1,21) zu.

Die Analyse der Differenzen in der relativen Häufigkeit der Blutgruppen A 0 bei den Sarkoidose-Patienten und der Kontrollgruppe ist in der Tab. I zusammengestellt. Nach der Woolf'schen Methode errechnete sich die relative Häufigkeit ( )

$$= \frac{\text{A Pat. 0 Kontr}}{\text{0 Pat. A Kontr}} = 1\,142$$

Der x^2-Wert nach der Formel

$$x^2 (n = 1) = \frac{(d - b)}{n_1 \; n_2 \; n_3 \; n_4}$$

beträgt 15,22, die entsprechende Irrtumswahrscheinlichkeit P ~ 0,0001.

Damit ist statistisch signifikant gesichert, daß Personen mit der Blutgruppe A eine um 14,2 höhere Wahrscheinlichkeit haben, an einer Sarkoidose zu erkranken, als Patienten mit der Gruppe 0.

schlechter und Altersgruppen hat ergeben daß die auf diese Weise gekennzeichnete Korperform unauffällig ist und dem Durchschnitt entspricht (Tab. IV—V)

In epidemiologischen Untersuchungen ist immer wieder darauf hingewiesen worden, daß die Landbevolkerung bevorzugt von der Sarkoidose betroffen ist. Diese Ansicht ist nicht stichhaltig Nur 35,5 ± 0 161 wohnen in einer Landgemeinde. Eine Bevorzugung bestimmter Berufsgruppen liegt ebenfalls nicht vor der Prozentsatz der in der Land und Forstwirtschaft beschäftigten Sarkoidose-Patienten (12,4 ± 3 82) liegt sogar unter dem der gleichen Berufsgruppe in der Bundesbevölkerung (16,8 ° ± ± 0,000058) Das Hauptargument für die Hypothese, der Typus bovinus des Tuberkelbacillus sei der Erreger der Sarkoidose, ist somit hinfällig

Tabelle III Haptoglobintypen bei Sarkoidose und im deutschen Sprachgebiet

		Hp 1—1 %	Hp 2—1	Hp 2—2 %	Hp¹	
Sarkoidose	absolut	66	189	137	0,4085	392
	%	16,7	48,3	35,0		
Deutsches Sprachgebiet	absolut	839	2 463	1 786	0,407	5 088
(Wichmann und Schleyer 1961)	%	16,5	48,4	35,1		

DISCUSSION

Dr Norodin Leonhardt (Acta med. Scand. Suppl. 416, 1964) who has studied the serum proteins in relatives of cases with systemic lupus erythematosus found hypergammaglobulinemia (> 1.90 gram per 100 ml) in 11.5 per cent. The control series showed such values in 3.8 per cent.

As sarcoidosis has been known to be followed by increased gammaglobulin values, Ingestad and Tryding at Lund have been collecting serum from siblings and children of known cases of sarcoidosis. The results are presented in table I. No deviations from the controls were observed in the serum protein electrophoresis. As you recall the series of sarcoidosis studied by Ingestad and Tryding as well as the results presented by Dr Renee Norberg showed only very slight abnormalities in the gammaglobulin levels in cases of sarcoidosis.

Table I. Serum γ-globulins in children and siblings of cases with sarcoidosis

Sarcoidosis				Relatives			
Group	No.	γ	ESR		No.	γ	ESR
I	36	1.11	16.3	Children	51	0.94	
II	40	1.34	20.1				
III	36	1.35	15.0	Siblings	83	1.04	6.4
IV	27	0.98	6.2				
Normal							
Adults	53	0.97	6.0				
Children	19	0.93					

Dr Scadding: I should like to draw attention to Lewis' (1961) study of blood-groups in London in relation to sarcoidosis and tuberculosis. Like Drs. Jørgensen and Worm, he found an excess of blood-group A and a deficit of 0 among sarcoid patients. Unlike them, he found an opposite tendency, though not of statistical significance, in patients with basal tuberculosis. His samples were considerably smaller than theirs, however.

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Dr Jonkerwez: Wenn eine Infektion eine entscheidende Rolle spielt, müßte man erwarten daß ein Ehepartner den anderen häufiger infizieren würde. Das ist jedoch nicht der Fall. Es gibt ja auch keine Epidemien oder gar Pandemien von Sarkoidose, kleine Endemien sind ebenfalls nicht bekannt. Das spricht dafür, daß die Konstitution und der genetische Hintergrund eine wichtige Rolle spielen. Trotzdem könnte es sich bei der Sarkoidose um eine Infektionskrankheit handeln. Die Infektion gibt gewissermaßen den letzten Anstoß zur Entwicklung der Erkrankung. Es könnte sich hierbei um die Tuberkulose handeln, jedoch auch um ein anderes Agens. Bei der Tuberkulose wissen wir durch die Zwillingsuntersuchungen von Diehl und von Verschuer von der wichtigen Bedeutung genetischer Faktoren an ihrem Zustandekommen. Aus meinen Untersuchungen läßt sich nichts aussagen über einen infragekommenden Erreger oder ein infragekommendes anderes Agens. Es ergibt sich aber aus ihnen unzweifelhaft, daß erbliche Momente von großer Bedeutung sind und eine wichtige Voraussetzung für das Zustandekommen der Sarkoidose.

TABELLE I Die relative Blutgruppenhäufigkeit bei der Sarkoidose und der Tuberkulose

	Anzahl der Stichproben	Gesamtzahl		Vergleich	Relative Häufigkeit	χ^2 (m = 1)	P Irrtumswahrscheinlichkeit	P der Heterogenität
		Patienten	Kontrollpersonen					
Sarkoidose	1	318	81 985	A : O	1 142	15 22	0,0001	—
Tuberkulose	10	4 505	19 883	A O	1 1360	12,19	0,0005	0,06

TABELLE II Verteilung der Rhesus-Gruppen bei 495 Sarkoidose-Patienten

	Rh	rh	n
Sarkoidose %	84 04	15,96	495
Kontrolle	84 60	15,4	448

Der analoge Vergleich der relativen Häufigkeit der Blutgruppen A B hat nur eine schwache Signifikanz zugunsten der Blutgruppe A ergeben Zwar besagt die Berechnung der relativen Häufigkeit nach der Woolf'schen Formel $x = 1\,419$ daß Angehörige der Blutgruppe A eine um 41,9 höhere Erkrankungswahrscheinlichkeit für die Sarkoidose haben als die der Blutgruppe B jedoch liegt di Irrtumswahrscheinlichkeit P ~ 0 02 bei einem χ^2 Wert (m = 1) von 5,169 recht hoch. Diese geringe Signifikanz ist vermutlich durch die geringen absoluten Zahlen in der Gruppe B gegenüber A bedingt.

Von klinisch-epidemiologischer Seite konnten die Häufung von Sarkoidose Fällen in Schweden mit seinen besonders hohen A Frequenzen und die spärlichen Meldungen über das Auftreten von Sarkoidose in asiatischen Ländern, in denen die Blutgruppe B um das zwei- bis dreifache häufiger ist als in europäischen Populationen, für eine Bl t gruppenabhängigkeit zugunsten der Gruppe A sprechen. Es ist allerdings kritisch zu berücksichtigen, daß allgemein aus asiatischen Ländern über alle selteneren und schwerer zu diagnostizierenden Krankheiten spärlichere Mitteilungen vorliegen.

Bemerkenswert ist in diesem Zusammenhang, daß in ähnlicher Weise auch bei der banalen Tuberkulose ein leichtes Überwiegen der Blutgruppe A von 13 6 % vorliegt, wie wir bei der Nachprüfung von 10 Stichproben aus der älteren Literatur mit moderner statischer Methodik — entsprechend den eigenen Untersuchungen bei der Sarkoidose — errechnen konnten (Tab. I)

Eine Abhängigkeit der Sarkoidose vom Rhesussystem (Tab. II) liegt im Gegensatz zu den AB0-Blutgruppen nicht vor Das Verhältnis von Rh zu rh stimmt mit der schon 1941 von Landsteiner und Wiener an 448 Personen festgestellten und später häufig bestätigten Verteilung in europäiden Populationen von 84 6 % und rh 15 4 sehr gut überein. $\chi^2 = 0{,}0544$ P ~ 0 78.

Desgleichen bevorzugt die Sarkoidose keine Individuen mit bestimmtem Haptoglobintyp (Tab. III) Der Vergleich von Hp 1 1/Hp2 2 im χ^2 Verfahren fiel entsprechend aus $\chi^2_{(m=1)} = 0\,000093$ p ~ 0 99

Zusammenfassend hat sich ergeben, daß Personen mit der Blutgruppe A eine rund 14 höhere Wahrscheinlichkeit haben, an einer Sarkoidose zu erkranken, als Personen mit der Blutgruppe 0. Es könnte sein, daß das Gen für die Blutgruppe A innerhalb des multifaktoriellen Systems, das der Sarkoidose zu Grunde liegt (Jorgensen) eine Rolle spielt.

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From the Department of Tuberculosis of University Medical School, Debrecen, Hungary

Sarcoidosis in Childhood

LÁSZLÓ MÁNDI

Up to the middle of the year 1963 we succeeded in collecting data on 86 sarcoidosis cases in Eastern Hungary. Among these there were 82 thoracic diseases and 4 cases of peripheral lymph-node sarcoidosis. Contrary to the reports in the special literature, the age range of 15—25 years was predominant in our material. There were 13 patients under 15 years of age. The frequent occurrence of sarcoidosis in childhood is the characteristic feature of this material. The youngest patients were a boy of 8 and a girl of 10 but the majority of them were either 13 or mainly 14 years of age. Thus, 1—2 cases occurred before puberty but mostly the disease is observed during puberty. According to this, neurohormonal influence also plays an important role in the emergence of sarcoidosis.

History

A study of the environment of the families of 13 of the children showed that tuberculosis had occurred only in one case among the members of a family.

If we consider our material from the point of view of the present discussion — whether BCG vaccination plays a role in the emergence of sarcoidosis in childhood — we can see that only 3 children were BCG-vaccinated in infancy. — They displayed a BCG cicatrix. Two were slightly tuberculin positive, and one showed a completely negative result. I am convinced that BCG inoculation is not a factor in the development of sarcoidosis, at least in so far as my cases are concerned.

Thoracic changes were observed	No. of cases
By pulmonary check-up (fluoroscopy)	10
After the appearance of erythema nodosum	2
By complaints (fever, cough)	1

These data show the decisive importance of mass X-ray survey in the detection of sarcoidosis in childhood.

X-ray morphology was as follows: Stage I of sarcoidosis, BHL syndrome, was observed in 9 cases. Stage II of sarcoidosis was noted in 4 cases, with extensive dissemination in the lungs, besides enlargement of the thoracic lymph nodes. Stage III and extrapulmonary sarcoidosis in childhood did not occur in this material.

As to the Mantoux tuberculin-test (1 : 100 dilution with Old tuberculin) it was positive only in 4 cases, and negative in 9 patients.

Results of biopsy	No. of cases
Axillary lymph node biopsy was positive	1
Praescalene lymph-node biopsies were positive	2
Tonsilla palatina and praescal. lymph-node positive	1
Excision of lungs and praescal. lymph-node positive	1
Excision of lungs and thoracic lymph-node positive	2
Thoracic lymph node by mediastinoscopy was positive	1
The excisions of Kveim test were positive	3
Total	11

The gastric lavages for culturing tubercle bacilli were negative in 12 cases and positive in 1.

The physiological value of the erythrocyte sedimentation rate was observed in 8 cases, it was slightly increased in 3 and greatly increased in 2 cases. The latter patients belonged to the disseminated group.

Biopsy showed that 11 out of 13 cases were positive. The specimens were taken from dif-

ferent organs. There were 2 cases where other diseases could be excluded on account of the clinical, X-ray and laboratory findings and the course of the disease.

During the course of the illness we observed the spontaneous recovery of 7 children. In 3 patients with the disseminated form of the disease, intense regression resulted from Prednisolone therapy with an antituberculous drug coverage as protection. The treatment of 3 children is still proceeding. Since most of the children had reached the stage of the BHL syndrome, they may recover spontaneously.

We will now present some of our cases very briefly.

The first case was a boy of 8. The enlargement of the thoracic lymph nodes was detected by mass X-ray survey on both sides of the hilus and in the paratracheal region. In the lungs there were rough interstitial reticula.

Family history and Mantoux tests were negative, without fever. The Kveim test was histologically positive results with the material sent by Putkonen. The treatment was symptomatic. After half a year considerable spontaneous regression was noted. After one year the enlarged lymph nodes regressed, but with fibrosis. The period of observation was three years.

The second case was a 14-year-old girl. Her history was exactly the same as that of the preceding case. BHL syndrome was detected by school X-ray survey. The Kveim test with Putkonen material and the biopsy were positive. Her treatment was symptomatic.

After one year a complete spontaneous recovery was achieved. Period of observation three years.

The third case was also a 14-year-old girl. Mass X-ray survey revealed BHL syndrome and many foci in both lungs. Family history and tuberculin test were negative. She had no complaints. The results of Daniels biopsy and of the biopsy of the bronchial mucous membrane were negative. The histological findings of the thoracic lymph nodes by thoracotomy and biopsy of the lung were sarcoidosis.

She was treated with prednisolone for a period of 3 months and considerable regression was observed. Since treatment was not continued at home, there was a relapse after half a year.

An almost complete recovery was attained by renewed prednisolone treatment for one year; the result seems to be permanent after three years observation.

The fourth case was again a 14-year-old girl, who was Mantoux positive. We found serious bilateral dissemination in the lungs. The histological findings in connection with the biopsy of the lungs and the excision of prescalene lymph nodes confirmed the diagnosis of sarcoidosis. From the gastric lavage fluid M. tuberculosis was cultivated. Treatment with prednisolone, isoniazid, and trecator resulted in complete regression. We consider that sarcoidosis and tuberculosis occurred simultaneously in this girl.

Summary

We observed 13 children with sarcoidosis in Eastern Hungary. Most of them were tuberculin negative. Diagnosis was confirmed by biopsy in 11 cases. Nine cases displayed the BHL syndrome and in 4 cases nodulous lesions of the lungs were also demonstrated.

In one year the BHL syndrome regressed spontaneously in all but two cases.

The more extensive changes required prolonged corticosteroid treatment.

It was demonstrated that thoracic sarcoidosis occurs in childhood at about the age of puberty. When conducting a mass-survey of children this should be borne in mind.

DISCUSSION

Dr CHAPMAN: Sarcoidosis in childhood is fairly familiar in the southern and southeastern states of the U.S.A. McGovern, I believe, added 9 cases of his own to the world literature, these derived chiefly from North Carolina. All of these cases were in Negro children. The same racial selection is evident in Kendig's report from Virginia. Nearly all were eight years of age or more, but all less than fourteen.

In the world literature there is a single case report of sarcoidosis in a baby 8 weeks old, but this case must be regarded as probably disseminated tuberculosis without caseation, a feature of tuberculosis in the very young.

The only case I myself have studied in 15 years of observation was a 4 year old Negro boy who came to the hospital with intense bilateral uveitis and enormous nodes in the mediastinum and neck. He was treated with corticosteroids for 18 months and has not relapsed during a follow-up period of two years.

Dr RAJO: It would be interesting to know if the tubercle bacilli which Dr. Mandi isolated were investigated further as to type and whether they were a human type strain or an anonymous type.

Dr MÁNDI: It was a human type strain.

Sarcoidosis and Pregnancy

K. H. Fried, Berlin, Germany

We did not find a complete series of X-rays for all our patients, which, of course, should not be taken during the early stages of pregnancy except for important reasons. Thus, it was impossible to clearly separate in all cases the influence of the gravidities from that of the lactation period. The evaluation is therefore more subjective than it would have been with a series based on experiments.

To draw a conclusion, however, seems to be justified since, out of more than 30 authors, who have dealt with this question the majority reported on only one, or at most, a few cases. Thus, these authors were even more subject to misjudgements or accidental results. As far as I am aware among those present, only Lofgren and Jorgensen have reported on a larger series of cases.

It is not possible to analyse all the available literature on the subject in a short period of time. Special attention should be paid to the papers of Franz, Wurm and Nitschke. The study of the literature reveals no particular trend: earlier reports often indicate gravidity as cause or report a later deterioration; during the last 10 years, reports indicating gravidity as a positive factor are predominant. With regard to the lactation period, opinions differ.

From 1948 to 1962 a total of 205 patients suffering from sarcoidosis were under observation; their date of birth ranged from 1885 to 1946. Consequently some of these patients had either not yet reached child-bearing age or were no longer fertile. Seven patients died during the observation period, three of these —according to autopsy—from generalized

Table I. Age in years of women patients when sarcoidosis was diagnosed

0 to under 10	1	30 to under 35	14
10 to under 15	9	35 to under 40	13
15 to under 20	36	40 to under 45	13
20 to under 25	28	45 to under 50	17
25 to under 30	23	50 and over	51

Table II. Organic manifestation at time of diagnosis of sarcoidosis

Localisation	Females
mediastinal lymph node stage (I) without complications	47
involvement of thoracic lymph nodes (I) and extrathoracic foci	19
involvement of thoracic lymph nodes and primary symptoms of pulmonary reactions (I—II)	13
involvement of pulmonary parenchyma with and without lymphoma (II)	56
involvement of pulmonary parenchyma (II) and extrathoracic foci	5
involvement of pulmonary parenchyma with primary symptoms of fibrosis (II—III)	5
pulmonary fibrosis (III)	12
pulmonary fibrosis (III) and extrathoracic foci	9
extrathoracic foci exclusively	19
total	203

sarcoidosis one patient died from bacillary tuberculosis; in three women-patients the connection between death and sarcoidosis could not be definitely determined as autopsy was not performed. Ninety-six cases were diagnosed through single or multiple biopsy, 3 by means of autopsy and 171 were treated either once or repeatedly as inpatients. In the rest of the cases diagnosis was established, as a consequence of out-patient observations over considerable period of time as during its course the disease displayed typical symptoms.

In all the cases we tried to obtain gynecological history but were unable to procure complete records for every patient. Probably an undetermined number of abortions ha to be taken into consideration, as some of the women were unmarried or widows.

Group I

Pregnancies before detection of sarcoidosis

In 44 women we found 62 normal pregnancies and 4 miscarriages. The influence of pregnancy, partus, or lactation period was not suspected as the cause of sarcoidosis, diagnosed at later stage. This statement can be made only with reserve as in 18 out of the 44 women sarcoidosis was diagnosed only in the form of thoracic manifestations in stage III or as skin-sarcoidosis. Influence of gravidity cannot be definitely excluded, since frequently extremely long latencies, without any subjective or objective symptoms, can be observed.

In 6 other women, 10 pregnancies and 1 miscarriage were ascertained the last pregnancy had been terminated at least one year before the detection of sarcoidosis. The diagnosis was made mostly during or shortly after the lactation period and, it may be assumed that these cases were—with one exception—"earlier" sarcoidosis stages. Hereby chronological, and perhaps even causal, connection is established between sarcoidosis and gravidity or lactation period.

Group II

Out of 12 women with active sarcoidosis, 8 had normal deliveries, 2 had premature deliveries with viable infants, and 4 had spontaneous abortions in various stages of their pregnancies.

One woman, who had had normal delivery while suffering from sarcoidosis and whose condition during the first gravidity clearly showed improvement, had also 2 legal surgical abortions. Interruption of pregnancy did not have any favourable influence on the recidivous symptoms in spite of constant steroid treatment and earlier tuberculostatic therapy the woman still suffers from florid lung sarcoidosis stage II—III.

Apart from this isolated case it can be stated that in 9 pregnancies, partus and lactation periods could not be established as exerting any influence on the course of sarcoidosis. On the other hand, four other pregnancies showed quite definitely favourable influence. Among these cases there were some patients who had previously been given tuberculostatic treatment without success, and one untreated case which spontaneously healed after delivery.

Group III

11 pregnancies were studied in 8 women, who were examined after healing or stabilisation of their condition. One pregnancy ended in an abortion, another in the same woman, as an extrauterinary pregnancy which was surgically removed all the remaining pregnancies ended normally. None of the cases showed any influence on the previous condition, particularly no recidivation could be observed.

Conclusions

1) A number of sarcoidosis cases were observed for the first time during lactation period or within 12 months after partus. However the diagnosis of this material was often quite accidental X-ray-examinations before conception were rarely available, so that no certain statement can be made as to causality or accidental coincidence, and calculation of statistical significance is pointless.

2) Florid sarcoidosis often shows improvement due to pregnancy or remains unaffected.

3) Definite deterioration in florid cases during lactation was not observed this is contrary to some reports in the literature.

4) Healed sarcoidosis, or stationary foci are generally not reactivated by gravidity or lactation period.

5) Interruption of pregnancy because of sarcoidosis without pulmonary cardiac, or renal insufficiency is medically pointless, and is probably even contraindicated This statement is analogous to recent findings on corresponding tuberculous stages and other infections, to which Franz and Wurm have drawn attention.

References

Abitol M. N Y St. J Med. 58 (1958) 1882.

Aikens, R. L., and Beckwith, O. J W Dis. Chest. 28 (1955) 580.

Altchek, A., Gaines, J A and Siltzbach, L. E. Amer J Obstet. Gynec. 70 (1955) 540.

Aykan, F and Juskowitz, N Dis. Chest 17 (1950) 544

Baas, N.A., and van Voorst Vader P J A. Ned. T Geneesk. 101 (1957) 1111

Berman, R. H. J Amer med. Ass. 117 (1951) 246.

Donaldson, S. W Tompsett, A. C., Grekin, R. H., and Curtis, A. C. Ann. intern. Med. 34 (1951) 1213.

Drube, H. Arztl. Wschr 12 (1957) 1021

Franz, G and Wurm, K. Tbk. Arzt 16 (1962) 696.

Fröhlich, M.: Zschr Tuberk. 113 (1959) 146.

Gallagher J P and Douglas, D H. Obstet. Gynec. 2 (1953) 590.

Heilmeyer L., Wurm, K. und Reindell, H. Klin. Tbk. 114 (1955) 46 Münch. med. Wschr 98 (1956) 145.

Holt, J F and Owens, W M. J Radiology 53 (1949) 11

Jorgensen, G (in Druck)

Krupp, Ph. J Amer J Obstet. Gynec. 73 (1957) 248.

Laczko, F Dermat. Wschr 105 (1937) 1370.

Lee, K. B. Beitr Klin. Tuberk. 120 (1959) 388.

Leitner St. Der Morbus Besnier — Boeck — Schaumann, Benno-Schwabe-Verlag, Basel 1942

Lindig, W : Zschr inn. Med. 7 (1952) 744.

Löfgren, S.: Beitr Klin. Tuberk. 114 (1955) 75.

Löfgren, S., and Lundback, H.: Acta med. scand. 142 (1952) 259.

Mayock, R. L., Sullivan R. D., Crenning, R. R., and Jones Jr., R. J Amer med. Ass. 164 (1957) 158.

Nitschke, N. Zschr Ges. inn. Med. 14 (1959) 518.

Nitter L. Acta radiol. (Stockh.) 54 (1953) Suppl. 105.

Nordland, M. Ylvisaker H. S., Larson, P and Reiff, R. Minnesota Med. 29 (1946) 166.

Reisfield, D. R. Amer J Obstet. Gynec. 5 (1958) 795 u. Bull. Sloane Hosp. Wom. N Y 4 (1958) 42.

Reisfield, D R., Yahr, C., and Laurenzi, G. A. Surgery 109 (1959) 412

Robinson, R. Med. J Australia 1950 II 959.

Rogers, J E. W and Netherton J Amer med. Ass. 155 (1954) 974

Russel, K. P Amer Rev Tuberc. 63 (1951) 603.

Schreuß, H. Th. Haut u. Geschlkh. 61 (1939) 641

Stubbe, W Beitr Klin. Tuberk. 102 (1949/50) 446.

Werner E. Tbk. Arzt 15 (1961) 263

Dr Siltzbach A few years ago I recorded observations on pregnancy in patients with sarcoidosis, i.e. 22 pregnancies in 21 patients (Chapter "Sarcoidosis" in Complications of Pregnancy" edited by A. F. Guttmacher and J. J. Rovinsky, Williams and Wilkins, 1960 pages 137—147). Six patients improved during seven pregnancies and 14 showed no substantial change. Only one patient suffered a temporary flare-up of cutaneous sarcoidosis during the last trimester. So it would appear that pregnancy itself had a distinctly favorable effect on the course of sarcoidosis. But, in the postpartum period relapses are common and, in some instances, sarcoidosis made its first appearance. Thus among the 21 patients, all of whom had normal deliveries, seven patients showed relapse within a few months of delivery and three more patients experienced their first attack of sarcoidosis within two months after delivery — phenomenon first stressed by Dr Löfgren. This rate of relapse in the postpartum period is certainly greater than one would ordinarily expect in the nongravid patient. Whatever favorable effect is exerted on the sarcoidosis process during pregnancy is obviously lost in the postpartum period.

Dr Scadding Probably because it was concealed in a paper on the general prognosis of pulmonary sarcoidosis, Dr Fried has missed my reference to the effects of pregnancy observed during a 5-year follow-up of 135 patients. Twelve pregnancies were observed in 10 women. During 4 of these pregnancies, shadows in the lungs cleared, only to return after delivery. Apart from this, the pregnancies had no discernible effect on the course of the sarcoidosis. All but one of the pregnancies resulted in a healthy child; in the remaining case, twins were still-born, and no reason for the still-birth was found.

Scadding, J. G. (1961) Brit. med. J. 2: 1165.

Dr Chapman It is a well-established fact that serum corticosteroids are elevated during pregnancy but the level differs in different women and varies during the nine months. These changes in levels of corticosteroids may be the pertinent feature of resolution of sarcoidosis during pregnancy but it is also possible they are not specifically involved.

Dr Vascular Was the tissue material obtained by D and C examined histologically for sarcoidosis? Does anybody have data of such examinations?

Dr Fried I could never get the result of histological examination of either placenta or abortion material of those sarcoidosis patients who had given birth or suffered from an abortion during the course of the disease. It is very likely that in the majority of cases the existence of the disease was unknown to the maternity hospital. According to information by the head physician of the Universitäts-Frauenklinik (Gynaecological Hospital of the University) of West-Berlin, no cases of sarcoidosis during pregnancy have consciously been observed there during the past ten years.

As far as I know there are in literature also only three reports on histological examinations of placentas. The results regarding sarcoidosis are said to have been negative.

Dr Israel Endometrial scrapings were examined in 4 of our patients, and hysterectomies were performed in 4 others; none showed granulomas.

Dr Siltzbach I reported one case of sarcoidosis of the uterus (Reference: Sarcoidosis of the Uterus. Albert Altchek, Joseph A. Gaines, Louis E. Siltzbach, American Journal of Obstetrics and Gynecology, Volume 70, 03, pages 540—547 September 1955). We have tried on one or two occasions to look for sarcoidosis lesions in the placenta but it is an enormous job to do histological sections of the entire placenta and we got nowhere with it.

BIOPSY ASPECTS OF SARCOIDOSIS

Moderator J G Scadding

From the Sarcoidosis Clinic, Jefferson Medical College, Philadelphia 7 Pennsylvania

Selection of Biopsy Procedures for Diagnosis of Sarcoidosis

Harold L. Israel and Maurice Sones[1]

A diagnosis of sarcoidosis should not be made on the basis of clinical and radiologic characteristics alone, since similar changes occur in patients with tuberculosis, histoplasmosis and lymphoma. Until standardized and stable Kveim test materials are developed, the demonstration by biopsy of epithelioid granulomas must be regarded as essential to the diagnosis of sarcoidosis.

An analysis of our experience with biopsy techniques in 329 patients with sarcoidosis is presented in Tables I II and III The diagnosis in each case was based on typical clinical and laboratory findings and at least on demonstration of epithelioid granulomas. A total of 553 tissue specimens had been obtained from the 329 patients.

Taking into consideration ease of performance as well as productivity cutaneous sarcoids, which occur approximately in 15 per cent of patients, represent the most convenient source for biopsy

Lymph nodes are palpable, on careful examination, in more than half of patients with sarcoidosis, and in 7 cases out of 8 will provide histologic evidence of sarcoidosis. Epitrochlear nodes are to be preferred cervical, inguinal and axillary nodes are worthy of biopsy when epitrochlear nodes are not palpable.

Subcutaneous nodules, lesions of the nasal or conjunctival mucosa and enlarged parotid glands provide other readily accessible sites for biopsy

In approximately 40 per cent of cases of sarcoidosis, no accessible abnormalities are present, or biopsies of these abnormalities have given negative results. If arthralgia or erythema nodosum are present gastrocnemius muscle biopsy is a simple and productive technique In the remainder one has a choice of a variety of methods lung biopsy, scalene fat pad biopsy and aspiration biopsy of the liver are most widely used.

Intercostal pulmonary biopsy performed under local or brief inhalation anesthesia is the technique of choice in patients who have disseminated pulmonary lesions. This method has proven extremely accurate in demonstration of sarcoidosis, and has the additional [illegible]tue of providing a histologic diagnosis in

[1] Abridgement of paper published in Archives of Internal Medicine 1964

This study was supported by grants from the American Thoracic Society and Pennsylvania Thoracic Society

TABLE I. Results of Biopsy of Accessible Abnormalities

	Number of Biopsies	Number Positive	Percentage Positive
Epitrochlear lymph nodes	15	15	100
Enlarged parotid glands	7	7	100
Nasal mucosal lesions	7	7	100
Subcutaneous nodules	3	3	100
Cutaneous lesions	66	58	88
Inguinal lymph nodes	25	22	88
Cervical lymph nodes	97	84	87
Axillary lymph nodes	63	51	81
Enlarged tonsils	5	4	80
Conjunctival lesions	4	3	75
Total	292	254	87

TABLE II. Commonly Used Biopsy Procedures in Absence of Accessible Abnormalities

	Number of Biopsies	Number Positive	Percentage Positive
Mediastinal lymph nodes	5	5	100
Lung	67	66	99
Liver	61	49	80
Scalene fat pad nodes	54	40	74
Gastrocnemius muscle	13	9	69
Bone marrow	10	3	30

TABLE III. Results of Miscellaneous Biopsy Procedures

	Number of Biopsies	Number Positive
Larynx	2	2
Abdominal nodes	2	2
Spleen	2	2
Stomach	1	1
Colon	1	1
Kidney	2	1
Pleura	2	1
Bone	2	1
Thyroid	3	1
Uterus	4	0
Endometrium	4	0
Normal conjunctiva	4	0
Bronchial mucosa	1	0
Testes	1	0
Total	31	12

cases that prove not to be sarcoidous. Intercostal pulmonary biopsy has been *in our experience* little more formidable a procedure than scalene fat pad biopsy and because of its greater productivity and greater specificity it is to be preferred in patients with pulmonary lesions.

In patients with hilar adenopathy and no parenchymal disease, the most *commonly* used procedures are needle biopsy of the liver and scalene fat pad biopsy. There is little to choose between the two in productivity, *in specificity* or *in safety* in experienced hands. Liver biopsy is perhaps preferable if this organ is palpably enlarged. Although a more formidable procedure, thoracotomy is equally safe and more definitive. The choice among these methods is reasonably made largely on the basis of the skill and experience available with these techniques in a particular hospital.

BIOPSY ASPECTS OF SARCOIDOSIS

Moderator J G SCADDING

From the Sarcoidosis Clinic, Jefferson Medical College, Philadelphia 7 Pennsylvania

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HAROLD L. ISRAEL and MAURICE SONES[1]

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An analysis of our experience with biopsy techniques in 329 patients with sarcoidosis is presented in Tables I II and III The diagnosis in each case was based on typical clinical and laboratory findings and at least one demonstration of epithelioid granulomas. A total of 533 tissue specimens had been obtained from the 329 patients.

Taking into consideration ease of performance as well as productivity cutaneous sarcoids, which occur approximately in 15 per cent of patients, represent the most convenient source for biopsy

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Intercostal pulmonary biopsy performed under local or brief inhalation anesthesia is the technique of choice in patients who have disseminated pulmonary lesions. This method has proven extremely accurate in demonstration of sarcoidosis, and has the additional virtue of providing a histologic diagnosis in

From the Departments of Pulmonary Diseases and Surgery, St. Göran Hospital, Stockholm, Sweden

Principles and Procedures for Obtaining Biopsies in Sarcoidosis

Sven Löfgren and Björn Skellman

It has been emphasized that the clinical criteria are of fundamental importance for establishing the diagnosis of sarcoidosis (6). However, in all cases of clinically suspected sarcoidosis we are anxious also to get a biopsy compatible with the clinical diagnosis.

Sarcoids of the skin and naso-pharyngeal mucosae are the most accessible specimens for biopsy. In sarcoidosis patients suffering from nasal obstruction, polyps of the sarcoid type are fairly often found in the nose; on gross examination yellow-brown in colour, on biopsy disclosing characteristic sarcoid tissue (3).

More important, however, are the skin sarcoids, and, especially, the cutaneous scar sarcoids. In the St. Göran material we have found them present in ten per cent of the sarcoidosis patients (4). As a rule, it is far easier to make a skin biopsy than a lymph-node biopsy and, consequently, recognition of scar sarcoids is very helpful in diagnosis.

The scar sarcoids are principally of two types. One group is formed by sarcoids that develop in old post-traumatic scars. Practically everyone has, for example, scars on his elbows or knees, which are the result of road accidents in childhood. If, twenty or thirty years later, pulmonary sarcoidosis occurs, in some cases there will appear a reddening and swelling of these scars, and biopsy will then disclose a picture of sarcoidosis. Through micro X-ray diffraction it was possible to establish that quartz particles were regularly present in scars of this type.

Postoperative scars constitute the other group. In the same manner as mentioned above, some of these scars will also become swollen if a generalized sarcoidosis develops. But, since, as a rule, the operations were performed when the patients were adults, the intervals between traumatization and scar swelling are usually decidedly shorter in this group: ten to twenty years.

The shortest intervals are seen in patients operated on during the stage of active sarcoidosis, for example, with a scalene node biopsy. Especially in cases of a generalized type, a local scar sarcoid may occur within a few weeks postoperatively.

Sometimes postoperative scar sarcoids have been misdiagnosed as keloids, but both clinically and histopathologically there is a great difference between them.

If a scar biopsy reveals a "foreign-body reaction," there is good reason to perform a pulmonary X-ray, which, in most cases, will disclose sarcoidosis. In our material, micro X-ray diffraction of the scar sarcoids of this type has regularly shown the presence of talc particles.

Summarizing, it may be stated that in all scar sarcoids examined we found foreign bodies, either quartz or talc. This appears to

Sarcoidosis is a systemic disease which involves many organs and tissues, so that there are few biopsy sites that will not in one case or another provide positive results. *One* can eventually obtain histologic evidence of sarcoidosis by a series of biopsies of relatively low yield: blind conjunctival biopsy, bronchoscopic biopsy, aspiration biopsy of the marrow, scalene fat pad biopsy, etc. We are increasingly impelled, however, to recommend, instead of a series of minor procedures, the *one procedure which is most likely to afford* a definite diagnosis. The following schema summarizes this approach.

		Primary Procedure
I	Cutaneous sarcoids present	punch biopsy
II	Subcutaneous nodules or lymph nodes palpable	excision
III	Erythema nodosum or arthralgia present	gastrocnemius biopsy
	(I, II, III absent, or primary procedures negative)	
IV	Pulmonary lesions present	intercostal pulmonary biopsy
V	Hilar adenopathy only	liver biopsy, anterior thoracotomy or scalene fat pad biopsy

To avoid misunderstanding, we would suggest that more distinct terminology should be used for lymph node biopsies, and this will be in agreement with the terms used by Dr Israel in the paper which he read this morning:

scalene lymph node biopsy, when nodes are palpated in the scalenus region before operation

scalene fat pad biopsy (instead of "Daniels operation"), when no lymph nodes have been found on palpation.

References

1. Carlens, E. Dis. Chest 36: 313, 1959.
2. Löfgren, S. & Lundbeck, H. Acta med. Scand. 142: 259, 265, 1952.
3. Löfgren, S. Ibid. 145: 424 463, 1953.
4. Löfgren, S., Snellman, B. & Nordenstam, H. Acta chir. Scand. 108: 405, 1955.
5. Löfgren, S., Snellman, B. & Stavenow, S. Nord. Med. 69: 409, 1963 (Swedish)
6. Löfgren, S. Proc. 3rd Intern. Conf. on Sarcoidosis. Acta med. Scand. Suppl. 425: 3, 1964.

COMMENT

Dr Kooij: In an interesting way the relationship between foreign bodies and scar swelling in sarcoidosis is illustrated by the following case. A man aged 32 years was tattooed 10 years previously with blue and red colours. In October 1962 some red parts became swollen and resembled clinically a sarcoid nodule. Such on histological examination showed a sarcoid structure.

At the same time an X-ray of the chest showed bilateral hilar adenopathy. A previous X-ray of the chest in 1959 had not revealed any abnormalities. Mantoux reaction 1:1000 negative.

Within a few months the skin as well as the lesion in the chest subsided considerably without treatment. An interesting fact is that only the red parts of the tattoo reacted and the blue parts not at all. This shows that a patient with sarcoidosis reacts only to one or a limited number of substances.

I am indebted to Dr. Th. G. Nedemann, Zaandam, for the permission to demonstrate this patient.)

be of interest from a theoretical point of view In this connection, however we do not wish to deal with this problem in further detail.

In order to join in today's discussion, we will also mention that, since about 15 years, we have been dealing, to some extent, with bronchial biopsies in cases of sarcoidosis. However in patients with hilar adenopathy alone the positive results have been rather scanty (2)

When sarcoids of the mucous membranes and skin are not accessible, the peripheral lymph nodes are the parts most available for getting a biopsy specimen. In cases of hilar adenopathy palpable lymph nodes are found, as a rule only in the supraclavicular region, close to the paratracheal lymph node chain, mostly on the right side. In cases of chronic or generalized sarcoidosis, sometimes a swelling of the lymph nodes occurs in other regions too, as Dr Israel reported. Probably his material includes more chronic cases than ours.

Much confusion exists in the terminology used for lymph-node biopsies. No doubt Daniels technique has meant a great advantage for obtaining lymph node biopsies in mediastinal processes. According to Daniels' original paper his method should be applied to mediastinal conditions where lymph nodes cannot be palpated in the scalenus region, and hence a fat pad is excised from this region, possibly containing small lymph nodes for histopathological examination.

We have the feeling however that many lung physicians use Daniels method in a wrong way Many patients with pulmonary processes are sent to a surgeon to have Daniels biopsy performed without a preceding thorough palpation. In spite of the fact that big lymph nodes were present, the fat pad excision has been negative

Of course, it is far more important to excise a distinct lymph node than an indistinct fat pad. For this reason, since many years there has been close collaboration at St Göran's Hospital between physicians and surgeons. We have found that, if the physician and the surgeon together examine the patient, it is possible to palpate enlarged lymph

TABLE I Percentage of positive histological diagnoses in 261 cases of clinically suspected sarcoidosis. From Löfgren, Snellman & Stavenow 1962 (5)

Method of operation	No. of cases	Histological diagnosis: Sarcoidosis	Other positive diagnoses
Excision of palpable lymph nodes	194	173 (89 %)	4 (2%)
Daniels op.	47	15 (32 %)	0 (0)
Mediastinoscopy	35[1]	32 (91 %)	1 (3%)

[1] 15 of these cases were first operated on with Daniels method and showed negative results.

nodes in 70 per cent of the cases with clinically suspect sarcoidosis. And, if lymph nodes are palpated, they will, as a rule, be easily found on operation the histological diagnosis was positive for sarcoidosis in 89 per cent (Table I)

When no lymph nodes are palpable, we used Daniels method to excise the fat pad in the scalenus region and scrutinized it for minute glands for histological examination. With the selection and procedure mentioned, the positive results obtained with this method were comparatively few only in 32 per cent was a histopathological diagnosis established among cases with clinically suspect sarcoidosis.

Because of this we have adopted Carlen's mediastinoscopical method (1) When using this method we succeeded in obtaining a positive histological diagnosis in 91 per cent of the cases of suspected sarcoidosis, where no lymph nodes were palpable in the scalenus region. Enlarged lymph nodes seen around the tracheal bifurcation on the X-ray pictures are very often the reason why sarcoidosis is suspected. With mediastinoscopy it is possible to get specimens from these glands and in most cases, the histological diagnosis is positive for sarcoidosis.

Frequency according to the macroscopic appearance of the bronchial mucosa

Normal mucosa: 12 cases. Positive biopsies 6 cases (50 %)

Mucosa with ordinary and slight changes: 56 cases. Positive biopsies, 26 cases (46.4 %)

Mucosa obviously damaged 3 cases. Positive biopsies: 3 cases (100)

Frequency according to the location on the bronchial tree

Positive biopsies on both sides	18 cases (51.4)
Positive biopsies on the right bronchi	11 cases (31.4)
Positive biopsies on the left bronchi	6 cases (17.2)

Aspect and location of the histopathological lesions in the thickness of the mucosa

In the bronchial mucosa the appearance of the histopathological lesion is identical to that in other organs including the asteroid bodies and the bodies of Schaumann or phylloliths. They are located at random in the thickness of the endobronchus. They are either very diffuse and confluent or strictly localized, consequently the necessity of multiple sections and cutting in series of each specimen.

Future of the endobronchial lesions of the pulmonary sarcoidosis

One fact should be emphasized: the persistence of significant microscopic lesions in the bronchial mucosa long after the X-ray image of the sarcoidosis has disappeared spontaneously or following corticotherapy. Out of 12 cases submitted to that control, the bronchial biopsy was positive in 9 of them during period of 8 months to 12 years after the disappearance of the X-ray findings. Two principal facts may be derived from these observations.

1) The histopathological changes of sarcoidosis in the bronchial mucosa seem to be permanent.

2) The bronchial biopsy may serve as retrospective identification and consequently may contribute to the diagnosis of the cases of extra-pulmonary sarcoidosis when the deep visceral localizations are inaccessible to the histopathological examination.

From Hôpital Bichat Paris, France

Bronchial Sarcoidosis

J Turiaf

Special reference to bronchial biopsy

All the bronchial layers are susceptible to sarcoidosis. In rare cases, stenosis occurs and appears on the X-rays through images of hypoventilation or atelectasis or emphysema, and dilatations revealed by the bronchographies. Histopathology of the autopsy specimens shows well the extension and severity of the specific peri- trans- and endobronchial lesions.

Clinically the endobronchial damages are the most interesting. They are accessible to the biopsy by bronchoscopy and often aid effectively the diagnosis of th localized forms of the mediastino-pulmonary sarcoidosis.

Sarcoidosis of the bronchial mucosa is in most cases without clinical symptoms or significant X-ray findings. It shows rarely a characteristic aspect in bronchoscopy but is frequently significant by histopathology.

This paper is the result of the investigations of 71 cases of sarcoidosis in the various stages of evolution. The data obtained from the bronchoscopy and the bronchial biopsy are as follows.

Bronchoscopy rarely shows characteristic signs.

Aspect of the mucosa

Mucosa obviously damaged	3 cases (4)
Mucosa inflamed or thickened but without obvious or significant changes	56 cases (79)
Mucosa of normal appearance	12 cases (17)

Locations of the visible changes: in most cases in the lobar openings, on the dividing carinas and the neighbouring area.

Bilateral localizations	36 (50.7 %)
Unilateral localizations	[1]23 (32 %)

[1] right side 18
left side 5

Bronchial biopsy It shows the precocity, frequency and scattering of the sarcoidosis lesions which are present in the bronchial mucosa even in the absence of macroscopically visible changes.

To insure that the bronchial biopsy has the best opportunity to be positive it should systematically be done even in bronchi of normal appearance and should affect the carinas of the lobars on both sides in such a way as to obtain 3 to 4 sections which will be cut in series. With this method our results are

Positive biopsies	35 cases (49.3)
Negative biopsies	36 cases (50.7)
Total	71 cases

Frequency according to sex

Men (37 cases)	
Positive biopsies	20 cases (54)
Women (34 cases)	
Positive biopsies	15 cases (44)

Frequency according to the stage of evolution of sarcoidosis

Stage I	Positive biopsies	9 cases (41)
Stage II	Positive biopsies	24 cases (52)
Stage III	Positive biopsies	2 cases (66)

Table I. Findings in 49 patients with sarcoidosis who underwent bronchoscopy (144 biopsies)

	Patients no.	Pos. bronch. biopsies	Per cent positive
Bronchial mucosa grossly normal	27	12	44
Bronchial mucosa thickened	22	18	82
Bronchial symptoms absent	37	20	54
Bronchial symptoms present	12	10	83
Hilar adenopathy	15	6	40
Hilar adenopathy with mottling	19	15	79
Mottling only	15	9	60

Table II. Results of Single Random Palatal Biopsy in 26 Patients with Sarcoidosis

	No. of patients	Positive palatal biopsies	% positive
18 less than 2 years	12	5	42
10 more than 2 years	14	5	36
Hilar adenopathy	15	6	40
Hilar adenopathy with mottling	7	3	42
Mottling only	2	0	0
Normal	2	1	50
Other mucosal sarcoids present	9	4	44
Other mucosal sarcoids absent	17	6	35

Sites of Endobronchial Involvement

The bronchus belonging to the right middle lobe was most frequently the site of granulomas. The wall of the bronchus to the right lower lobe and the wall of the left main bronchus were also common sites. Radiographically, the finding of coarse streaks extending into both lower lung fields and middle zones, infrequently accompanied by atelectasis, was a good clue to the possible presence of sarcoidosis of the bronchi.

In short, sarcoidosis of the bronchus without symptoms is more common than had been realized and the technique of multiple random bronchoscopic biopsies is helpful, especially in the chronic stages of pulmonary sarcoidosis. Sixty per cent of patients with chronic pulmonary sarcoidosis demonstrate bronchial granulomas at a time when scalene fat-pad biopsy, mediastinoscopy and Kveim test are less likely to yield positive results. Because of its safety and simplicity bronchoscopic biopsy should be undertaken before open lung biopsy is performed since a positive result may show that lung biopsy is unnecessary.

Random Palatal Biopsy

Our experience with random bronchial wall biopsy has led us to a similar approach with respect to the mucosa of the hard palate. Dr Lester R. Cahn, Oral Pathologist to The Mount Sinai Hospital, *suggested this technique* after we had failed to find any granulomas in 7 patients who had random biopsies of the nasal mucosa in the absence of nasal symptoms.

Palatal biopsy is performed under local nerve block anesthesia. We remove a single, deep 5 mm core of palatal mucosa and submucosa employing a Hayes Martin drill biopsy punch, the same instrument with which we perform Kveim test biopsies. The biopsy of the hard palate is made in an area just anterior to the line where the hard palate and soft palate join. The tissue is obtained from either side of the midline. Minor bleeding occurs but this is controlled with Gel Foam powder if needed. The palatal biopsy area usually heals within two weeks. Because the palate always looks normal to the naked eye in patients with sarcoidosis, a truly random biopsy specimen is obtained in this procedure.

From The Mount Sinai Hospital, New Y rk, N Y

Random Biopsy of Bronchial and Palatal Mucosa in the Diagnosis of Sarcoidosis

LOUIS E. SILTZBACH and LESTER R. CAHN

My colleagues at The Mount Sinai Hospital and I have for some years been employing multiple random bronchial wall biopsies for the diagnosis of sarcoidosis (1 2) This report is a summary of our present-day views of the value of bronchoscopic biopsy in the diagnosis of sarcoidosis. In addit on, reference will be made to some recent experience with a new technique, random palatal biopsy which we have performed in 26 patients having sarcoidosis. It was thought that palatal biopsy might afford a simple and easily accessible approach to organ biopsy corroboration.

Regarding bronchial wall biopsy our technique is as follows

When the bronchial mucosa presents a normal appearance, four or five specimens are removed from areas adjacent to the spurs of major bronchi and from the carina. Any mucosal area showing thickening also is biopsied. Removal of several bronchial wall specimens appears to be no more difficult or dangerous than remov ng a single specimen. The finding of organized, noncaseating epithelioid cell tubercles occasionally with giant cells but without stainabl acid-fast bacilli is considered compatible w th the diagnosis of bronchial sarcoidosis.

We have performed 62 bronchoscopic biopsies in 49 patients having sarcoidosis, removing in all 144 specimens for histological examination (Table I) How did the results of bronchial wall biopsy correspond to the naked-eye appearance of bronchial mucosa?

We found that 27 patients or a little more than half of those bronchoscoped exhibited a normal-looking bronchial mucosa while 22 patients showed mucosal thickening, occasionally with narrowing of the bronchial lumen. No mucosal ulceration was encountered Two patients displayed warty granulomas and yellow plaques protruding f om the mucosal surface of a large bronchus.

Among the 27 patients with normal looking bronchi, 12 or 44 % showed granulomas on random biopsy The frequency of granulomas rose to 82 % when the bronchial mucosa ppeared thickened to the naked eye. In the biopsies of patients with bronchial symptoms such as wheezing, stridor hemoptysis and persistent cough, granulomas were found in 10 of 12 instances—or 83 But again, and this is perhaps more important, 54 of patients w thout significant bronchial symptoms also showed endobronchial granulomas.

As for the chest X ray patients in the early phase of intrathoraci sarcoidosis whose film showed hilar adenopathy w thout pulmonary opacities, bronchial wall granulomas were found in 8 of 15 or 40 When pulmonary shadows were present along w th hilar adenopathy 15 of 19 patients or 79 exhib ted abnormal bronchial biopsies. Even after hilar adenopathy had ubsided and pulmonary opacities alone persisted, 9 of 15 or 60 still showed granulomas.

palatal biopsy will prove to be In the diagnosis of sarcoidosis. These were selected patients since 19 of the 26 who underwent palatal biopsy had already had diagnosis of sarcoidosis corroborated by an organ biopsy Kveim test or both. But it is of some significance that among 7 patients without previous organ-biopsy confirmation of the diagnosis of sarcoidosis, 4 of these 7 patients exhibited palatal granulomas as the first support for the diagnosis. Random palatal biopsy might well be performed whenever more usual sources of biopsy confirmation are not productive.

References

1. Friedman, O. H., Blaugrund, S. M. and Siltzbach, L. E. Biopsy of Bronchial Wall as an Aid in the Diagnosis of Sarcoidosis. JAMA 183: 646—650, 1963.

2. Siltzbach, L. E., and Blaugrund, S. M. Sarcoidosis of the Mucosa of the Respiratory Tract. Annals of Otology Rhinology and Laryngology 72: 923, (Dec.) 1963.

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Fig 1 Specimen removed at random from mucosa of hard palate of a patient with sarcoidosis showing noncaseating epithelioid cell granulomas just beneath the squamous epithelium.



Fig. 2. Specimen removed at random from mucosa of hard palate of patient with sarcoidosis showing noncaseating epithelioid cell granulomas replacing some of the mucous glands.

Twenty-six patients having sarcoidosis have had random palatal biopsies and 10 or 38 % showed noncaseating granulomas without stainable acid fast bacilli. In only 2 specimens of the 10 which were read as positive was serial sectioning necessary to find the granulomas. In 8, they were found in routine slide preparations. "Control" palatal biopsies were also carried out in 12 patients without detectable evidence of sarcoidosis and none of these specimens showed any granuloma or other abnormality.

Table II shows that granulomas occurred in the palatal specimens of patients with sarcoidosis at about the same frequency whether the patient is in the subacute or chronic phase 42 and 36 respectively. Nor is there any important variation in relation to the chest X-ray patterns of these patients. However it is of some interest that positive findings in the palate were almost as common among patients who do not have mucosal lesions elsewhere as among those who do.

The noncaseating epithelioid cell granulomas occur beneath the squamous epithelium (Fig 1) or are embedded more deeply among the mucous glands (Fig 2). The mucous glands at times undergo considerable atrophy and may be almost entirely replaced by granuloma and scar tissue.

On occasion the granulomas occur just beneath the duct epithelium causing the epithelium to bulge and to become thinner. This phenomenon of a mucosal bulge has also been seen by us in the walls of bronchi and in lacrimal ducts. It would seem, then, that when an epithelioid cell granuloma is found to protrude into the lumen of a duct and the surface epithelium thins but does not ulcerate, one should think first of sarcoidosis.

This series of 26 patients does not show how useful a diagnostic measure random

TABLE I. Age when sarcoidosis was discovered

	Men			Women		
	< 30 years	30—50 years	> 60 years	< 30 years	30—60 years	60 years
Stage I	6	4	0	0	13	4
Stage II	2	5	1	3	12	4
Stage III	1	11	1	0	5	1
	9	20	2	3	12	4

TABLE II. Macroscopic findings in bronchi in sarcoidosis

	Mucosal swelling	Partial or total stenosis of bronchi	Parallel-running cords like broom	Yellow-white plaques	Sarcoidosis suspected
Stage I 27 cases	24 = 89	6 = 22	9 = 33	3 = 11	15 = 56
Stage II 27 cases	25 = 93	8 = 30	16 = 59	8 = 30	23 = 85
Stage III 19 cases	16 = 84	7 = 37	6 = 32	5 = 26	8 = 42

TABLE III. Results of bronchial biopsies

	Granulomatous lesions	Chronic bronchitis	Negative	Bronchoscopy not performed
Stage I	4 = 15	12	11	0
Stage II	12 = 44	10	5	0
Stage III	3 = 17	10	5	1

able. Signs of bronchial perforation have never been seen. In rather many cases, about two thirds of the material, the macroscopic picture of the mucosa ga[ve] strong suspicion of the occurrence of sarcoidosis, mostly in stage II.

The bronchial biopsy was positive in 26 per cent of cases, the highest frequency being in stage II, 44 per cent, see Table III. The result is rather modest as compared with that of Schenegle and coworkers, but it is probably due to the fact that biopsy was only performed on tissue taken from one place. In one case the biopsy was positive despite normal mucosa. I[n] no fewer than 60 per cent of the negative biopsies the report from the pathologist stated that there was chronic bronchitis of varying degree. These attendant chronic inflammatory mucosal lesions seem to be more common and pronounced in pulmonary sarcoidosis with parenchymal lesions.

Bronchography is of great interest despite or rather owing to, the strikingly insignificant findings. I[n] stage II with widespread parenchymal infiltrations, the bronchography is for the most part quite normal. Sligh[t] bronchial deformations, sometimes with unimportant bronchiectasias, may be seen, in very rare cases also bronchial stenosis, then generally of the middle-lobe bronchus. In stage III there are more often bronchographically demonstrable lesions, though these are scarce

From Kolmårdssjukhuset, Sweden

Bronchial Involvement in Pulmonary Sarcoidosis

I Ståhle

It is only in recent decades that writers have begun to throw light upon the occurrence and importance of bronchial lesions in different pulmonary diseases. It is thus not surprising that our knowledge of bronchial sarcoidosis is still slight, and that the occurrence of these bronchial lesions is supposed to be a rarity In 1962, Spencer writes in his book Pathology of the lung" merely that occasionally sarcoid lesions are found in the walls of the larger bronchi and when they heal they may result in bronchial stenosis How ever it is a fact that the bronchi are rather often involved in pulmonary sarcoidosis. But these lesions are clinically silent and are easily overlooked if bronchological examinations are not performed The state of the bronchi in sarcoidosis has nevertheless been discussed by several authors in France Germany the United States and other countries. Schieslle and coworkers have presented what is so far the largest material of sarcoidosis in connection with which bronchial biopsy has been performed. They got a positive biopsy in 55 per cent of 302 cases, and the highest occurrence was found to be in stage II (78) lower in stages I and III 40 and 50 per cent respectively They point out that biopsy ought to be carried out in all cases of sarcoidosis, even if the bronchial mucosa seems to be normal, and that biopsy samples ought to be taken from several places in the bronchial tree

The histo-pathological finding in bronchial sarcoidosis is rather like that in bronchial tuberculosis, and this in such a high degree that in Sweden pathologists are of the opinion that a diagnosis ought not to be put if the occurrence of acid-fast rods is not proved. The diagnosis of granulomatous lesions is given and it is the clinician who has to weigh this report against other clinical findings and then to put the diagnosis. The lesions are lying in the submucosa as ball-formed nodules of epithelioid cells with giant cells, but without necrosis. In the adjacent tissue there is generally a non-specific inflammatory reaction of the type chronic bronchitis"

My own material is rather modest, consisting of 73 cases, see Table I. The age-group 30—60 years is the predominant one, and the age-group < 60 years at the time of discovery of the sarcoidosis is also astonishingly high. Sarcoidosis does not seem to have such a pre-eminently early beginning as is generally believed. In 12 women and 1 man sarcoidosis began with the BHL-syndrome combined with erythema nodosum. 64 per cent of the cases were tuberculin positive on discovery more often in stages I and II 70 per cent, less in stage III 47 per cent

In all these cases a bronchoscopy was performed, and in all cases except one also bronchial biopsy The occurrence of the observed bronchial lesions is shown Table II Generally there is a mucosal swelling of varying appearance and intensity most easily observed at the edges of the large bronchi One can not infrequently observe fine parallel-running vessels like a broom. These are most clearly visible at the anterior and upper edges of the upper-lobe bronchi Local partial stenosis of bronchi may occur and flat yellow white plaques are sometimes observ

From the Thoracic Clinics, Karolinska Sjukhuset, Stockholm, Sweden

Biopsies in Connection with Bronchoscopy and Mediastinoscopy in Sarcoidosis
A Comparison

Erik Carlens

The symptoms manifested in cases of sarcoidosis may as we know vary considerably. One may frequently find big intrathoracic changes without the patient showing any symptoms at all. In other cases there may be a severe tickly cough, and one might then suspect the existence of bronchial changes.

In order to ascertain to *what extent bronchial* changes exist, we have in 56 cases of proven sarcoidosis performed biopsies from the bronchi. In order to get a picture of the whole bronchial wall, the biopsy has always been carried out from a septum at a bronchial bifurcation. The results may be seen in the table. From these 56 cases it seems to emerge that in stage 3 it is relatively often possible to demonstrate microscopic changes in the mucous membrane, in stage 2 more rarely and in the initial stage with only hilar nodes we have not been able to show microscopic changes in a *single* case. *In stage 2 we have* not been able to show any connection between macroscopic changes in the mucous membrane and the possible existence of tickly cough.

It is now known that the tubercle-like changes in cases of sarcoidosis are irregularly scattered in both lung parenchyma and bronchial mucous membrane. Thus in cases belonging to the stages 2 and 3 it is probable that one could always show microscopic lesions in the mucous membrane if one performed a sufficiently large number of biopsies. This, however, meets with certain practical difficulties. We have as a rule been content with 3 biopsies, generally one from the main carina and 2 from other places. The best chance of being able to verify the diagnosis microscopically appears to be to make a biopsy in macroscopically changed bronchi. The increased knowledge of microscopic bronchial lesions has during recent years resulted *in bronchoscopic biopsy being recommended* to ever more patients.

If there are clinical signs of bronchostenosis, bronchoscopy is of course always indicated, and if it is caused by sarcoidosis, it seems frequently to be possible to verify the diagnosis. In 44 patients in stage 1 or 2, however, where there were no signs of bronchostenosis, we were in only 6 cases, or about 15 per cent, able to verify the diagnosis with bronchoscopic biopsy. If there is not at the same time any other indication for bronchoscopy we think the result must be considered *poor in comparison with other diag*nostic methods. Of these, probably biopsy from *palpable* glands in the neck is the simplest procedure, and is according to unanimous testimony able to verify the diagnosis in nearly 100 per cent. Where, on the other hand, there are no palpable glands, Daniels' prescalene node biopsy has proved to be very valuable and has in long series given positive results in up to 60 per cent of suspected sarcoidosis. Here, however, the reports vary and even higher figures have been given often due to the fact that the extirpation of *palpable* glands has not been delimited from Daniels' operation. When they con-

ly characteristic, and of the type generally observed in pulmonary fibrosis. One may see bronchial deformations of bronchitic type, sometimes with bronchiectasias, or more or less pronounced stenosis. The picture is quite other than that in pulmonary tuberculosis, where there is generally stenosis of peripheral bronchi at the site of the parenchymal lesions, giving the picture of so-called naked filling. This fact is of interest in the differential diagnosis between sarcoidosis and tuberculosis. In chronic pneumonic infiltrations the bronchographic picture is also quite a different one.

The occurrence of bronchial tuberculosis has great importance in pulmonary tuberculosis and is a common finding especially in the peripheral bronchi. Bronchial sarcoidosis is not at all of the same importance, if, indeed any. During the course of pulmonary sarcoidosis there are undoubtedly in most, perhaps all cases during some phase submucosal nodules with sarcoidal tissue in the larger bronchi. Whether the peripheral bronchi are affected is far more uncertain. However it is important that in pulmonary sarcoidosis a thorough bronchological examination be performed. In many cases the malady may be verified in a way very easy for the patient. The state of the bronchi clearly supports the opinion that the etiology of pulmonary sarcoidosis is different from that of tuberculosis, at least in most cases.

Summary

Bronchological examination with bronchoscopy and in several cases also bronchography has been performed in 73 patients with pulmonary sarcoidosis. It has been shown

that bronchial lesions in sarcoidosis seem to be rather common, especially in stage II,

that the bronchoscopic finding may be characteristic and may contribute to the diagnosis,

that biopsies of the mucosa from several parts of the bronchial tree are of great diagnostic value and ought to be performed in every case where sarcoidosis is suspected, even if the mucosa appears to be normal,

that the bronchographic finding is clearly distinguished from that in pulmonary tuberculosis and is rather often quite normal

and that bronchial sarcoidosis rarely gives rise to deterioration of the malady.

References

Adamson C. A. & Carlens, E. Acta Chir Scand. suppl. *245* 43—46 1959
Benedict, E. B. & Castleman, B. New England J Med. *224* 186, 1941
Chrétien, J Path. Biol. *7* 1905—1911 1959.
Citron, K. M. & Scadding J G Thorax *12*: 10—18, 1957
Hirschfeld, J H. & Kress, M. B. Dis. Chest. *39*: 284—291 1961
Huzly A. Atlas der Bronchoscopie. Georg Thieme Verlag Stuttgart, 1960.
Jullien, J L. Ouest. Méd. *12*. 581—584 1959.
Kalbian, V V Thorax *12*. 18—23, 1957
Kampfer R. Tub. Arzt *13*. 261—274 1959.
Lofgren, S. Acta Tub. Scand. suppl. *45*. 15—18, 1958.
Lofgren, S. Ibid. Suppl. *45*. 19—22, 1958.
Lofgren, S. Am Rev Resp. Dis. *84* 17—19 1961
Rubin, E. H. The lung as mirror of systemic disease. Charles C. Thomas Publ. Springfield, Illinois. 1956.
Rubin, E. H Thoracic Diseases. W B. Saunders Company Philadelphia and London. 1961
Schuerde W Wurm, K. & Reindell, H. Munch. Med Wschr *103* 726—730, 1961
Simonin, P Lamy P Briquel, P Hutin, R. & Anthoine, D Rev Méd. Nancy *85*. 724—734 1960
Spencer H. Pathology of the Lung Pergamon Press, London, 1962
Turiaf J Besnier Boeck-Schaumann disease. Excerpta Medica (Capitula Selecta) 11/3. 129—133, 1958.
Uehlinger E. A Am. Rev Resp. Dis. *84/II* 6—13, 1961

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Observations on the Significance of Mediastinoscopy in the Diagnosis of Sarcoidosis

JORMA PÄTIÄLÄ

Mediastinoscopy performed in the form presented by Carlens in 1957 has been in use in Finland since 1958 in the diagnosis of intrathoracic diseases. Particularly it has been considerable aid in the diagnosis of sarcoidosis.

My series consists of twenty-five cases of sarcoidosis in which the diagnosis was based on mediastinoscopy. There were thirteen women and twelve men. The mediastinoscopy was performed in all cases by Dr. A. J. Seppälä.

According to the X-ray findings the cases were distributed as follows: In twenty cases the observed changes were restricted to the hilar region, being bilateral in seventeen and unilateral in three cases. In four cases there were — in addition to enlarged hilar nodes — peripheral bands and spots. Only in one case there were extensive peripheral changes and fibrosis.



Fig. 1

It is interesting from a diagnostic standpoint that scalene node biopsy was done in nine cases, or in over one-third of the series, but the findings were negative in all instances. Bronchoscopy had been performed in sixteen cases. The bronchial mucosa appeared normal in all cases and a biopsy specimen was taken in one case only. No sarcoid tissue was seen in the biopsy.

A comparison of the findings made by scalene node biopsy and mediastinoscopy is shown in the table.

Although the presented series is small, it gives further evidence clearly supporting the observation emphasized by Carlens (1959) and in my country by Seppälä (1959) that mediastinoscopy has made a diagnosis of sarcoidosis possible in cases where a scalene node biopsy from the neck has given negative findings. The reason is that the scalene node biopsy can provide us with a sample from only the peripheral lymph nodes, whereas mediastinoscopy has direct contact with the hilar nodes of the lungs. No complications occurred in connection with mediastinoscopy in our series. The following case illustrates the above-mentioned facts.

TABLE I Bronchoscopic biopsy (from 3 different places) in 56 patients with verified sarcoidosis

Stadium	Number of cases	Sarcoid. lesion
1	9	0
2	35	6
3	12	5
Mediastinoscopy	123	118 (96)

sistently excluded all palpable glands, Lofgren & Snellman obtained positive results in their material with Daniels operation in only 32 per cent of the cases. With mediastinoscopy the same writers, using the same principle of selection, obtained a positive diagnosis in 91 per cent of cases.

During the last 5 years we ourselves have, in 123 cases with a reasonable suspicion of sarcoidosis, been able to verify the diagnosis with mediastinoscopy in 118 cases, which corresponds to 96 per cent. In a large number of these cases other diagnostic methods had been tried before mediastinoscopy was undertaken. Mediastinoscopy seems thus to be the most reliable method, but appears on a superficial view also to be the most elaborate procedure. However we have not experienced any complication whatsoever in our cases. With Daniels operation, however complications have been reported, and the scar on the neck is as a rule bigger than after a mediastinoscopy and in our limited material we have twice experienced moderate haemorrhages in connection with biopsies from the bronchi. This risk will of course increase in proportion as one increases the number of biopsies to get a higher percentage of positive results.

With mediastinoscopy one obtains also in other respects a higher degree of diagnostic certainty. The histological picture in small pieces of bioptic tissue may sometimes be difficult to distinguish from the picture given in cases of tuberculosis. A guinea pig test from the same material is therefore valuable. From small biopsies it may be difficult to do this, but from the large glands in the mediastinum one always gets sufficient material also for this examination.

To summarize, I will explain the principles according to which we act where it is a matter of verifying the sarcoidosis diagnosis. In all cases we look for a palpable node in the neck. In this way one can verify the diagnosis in a very high percentage of cases. Where bronchostenosis is suspected we always perform a bronchoscopy with biopsy. If this is not successful, or if one cannot palpate any glands, we often perform a mediastinoscopy direct. In those cases in which we have considered it indicated, we have often performed a bronchoscopy at the same sitting as the mediastinoscopy. In this way one saves time and can with a great degree of reliability both confirm and correct a clinical suspicion of sarcoidosis.

References

1 Lofgren, S Snellman B & Stjernberg S. Biopsier for diagnos vid sarkoidos. Nord. Med. 69 409 1963 (Swedish)

2 Skinner D B. Scalene node biopsy Risks and indications. New Engl. J Med. 1324 ?68 1963.

3. Carlens, E. Mediastinoscopy A method for inspection and tissue biopsy in the superior mediastinum Dis. Chest 343 36 1959.

4 Maassen, W Die Mediastinoskopie Biopsie nach Carlens) eine neue diagnostische Methode bei Thoraxerkrankungen. Deutsche Med. Wschr 2004 87 1962

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The Value of Lung Biopsy in the Diagnosis of Pulmonary Sarcoidosis

L. Levinsky, F. Řehák and N. Žáková

At the 1st University-Clinic for Tuberculosis in Prague we have succeeded in collaboration with the 2nd Surgical Clinic, in simplifying the technic of lung biopsy so that it can now be used for nearly all patients with X-ray evidence of disseminated pulmonary lesions, where clinical and laboratory investigations failed to establish the diagnosis. By this method it is possible to obtain tissue for lung biopsy even in patients with severe dyspnoea.

Technic

The surgical procedure is carried out under general anaesthesia with oxygen and nitrous oxide, administered through the endotracheal tube after routine pre-operative medication and induction (Dolsin, atropine, thiopental, succinyl iodide). The patient is placed on the operating table in the supine position with slight elevation of that side of the thorax which is to be operated on. The approach and the site of the opening of the thorax are chosen according to the localisation of the most severe pulmonary lesions. In most cases we use the submammary or the anterior axillary incision at the level of the third up to the fifth rib. An incision from five to six centimeters in length has proved satisfactory. After an incision of the skin and subcutaneous tissues we divide sharply or bluntly the major pectoralis or the lateral serratus muscle and penetrate as far as the costal periosteum. We never enter the thorax through the intercostal space or resect a rib. The periosteum is sharply divided and the upper half and the posterior surface of the rib are denuded. After this, the pleura is subcostally sharply incised. By this approach a sufficient exposure of the lung is obtained, permitting palpation with two fingers and an excision to be made.

The extent of the wedge excision is marked by two fixing sutures in the range of 2—3 ccm in the non-collapsed tissue. The lung is closed with single or a continuous suture. Sometimes the edges of the incision are sutured with a single buried suture. This technic may be used also in limited pleural adhesions or even in total symphysis of the parietal and visceral pleura. From the thoracotomic opening extrapleural pneumolysis is carried out, to a small extent, and in the extrapleural space thus obtained, it is possible to make an excision of pulmonary tissue, similarly to that made in a free pleural cavity. The closure of the chest wall is easy and rapid. After the re-expansion of the lung, the periost, with the intercostal muscles, is sutured back to the rib so as to be air tight, and the integrity of the chest wall is restored. We neither insert a drain, nor instillate antibiotics into the pleural cavity. The whole surgical procedure does not last longer than twenty to twenty five minutes and the patient is allowed out of bed on the first postoperative day.

Fig. 1 shows a postoperative scar two weeks after biopsy in a 16-year-old girl, M. J. The scar is only three and a half centimeters. In this case a submammary incision was not possible, as most of the pulmonary lesions were situated subaxillarily in the upper lobe.

To date, this procedure has been performed in 40 patients. In 8 of these it was helpful in overcoming the difficulties associated with the differential diagnosis of pulmonary sarcoidosis. These 8 patients were divided into three groups, as follows:

Case reports

Group I

Patients with radiological diagnosis of disseminated pulmonary tuberculosis. All of them had, however, a negative tuberculin reaction. At no time were tuberculous mycobacteria

TABLE I

X-ray findings	No. of cases	Scalene node biopsies, all negative	Mediastinoscopies, all positive
Changes limited to hilar region	20	8	20
Enlarged hilar nodes and slight peripheral changes	4		4
Enlarged hilar nodes and extensive peripheral changes and fibrosis	1	1	1

A 32 year old unmarried woman, laboratory worker entered the Department of Pulmonary Diseases, University of Helsinki April 1st, 1963 in a good state of health with normal temperature. *Sedimentation rate mm. The serum calcium was* 9.8 mg per cent. Blood counts normal. The Mantoux test (10 TU) was negative. Chest x-ray showed enlarged hilar nodes (Fig 1). Scalene node biopsy liver biopsy and bronchoscopic biopsy negative. Mediastinoscopy From a group of lymph nodes in the angle between trachea and right main bronchus a small fragment was removed for examination. PAD Sarcoidosis.

References

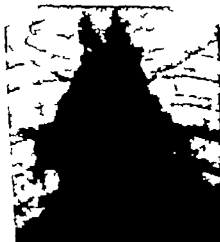
Carlens, E. Dis. Chest 4 343, 1959.
Seppälä, A. J Duodecim 75, 435 1959.



Fig 2. a. MUK P born 1912



b. Mi-Fo 5146, 135 x.





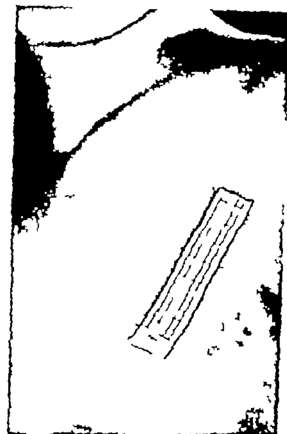


Fig 1 M J Postoperative scar tw weeks after biopsy

demonstrated and ant tuberculous drugs failed to influenc th pulmonary lesions.

Case 1

SRB. \ born *1919 female, shop assistant* in a food shop. The pulmonary lesions were detected by mass photofluorography in the year 1954 The diagnosis of chronic pulmonary tuberculosis was established. Antituberculous drugs wer neffective

Lung biops (18 June, 1959 \ 53 59 *Dispersed epithelioid nodules, fusing in some places* nd, in other places showing signs of fibrous transformation numerous gia t cells nd isolated tar shaped inclusion bodies were present in the cytoplasm. \ areas of aseation were found. The histologic pattern correspond t that observed in sarcoidosis. *After* ortisone herapy lone. the patient chest \ ra film showed clearing of the disseminated pulmonary lesions.

Case 2

MUK. P born 1912 female, an office worker The first symptoms developed in September 1959. In J ly 1961 th patient s chest X-ray film showed changes, which were interpreted as subacute disseminated pulmonary tuberculosis *(Fig 2 a)*. Antituberculous drugs, both alone and in combination w th prednisone, failed to influence the pulmonary process.

Lung biopsy (27 March 1962, *B 1409/61)* Numerous conglomerated epithelioid granulomas w th *many giant multinucleated Langhans* cells containing in some places small vacuoles. In some epithelioid granulomas signs of fibrous transformation were seen (Fig. 2 b) *No areas of caseation* were found. The histologic pattern confirmed the diagnosis of sarcoidosis.

Case 3

JEHL. \., born 1925, labourer diabetic, was *admitted* to our clinic on 24 June 1962. On roentgenography of the chest, small nodules were observed, which fused in some places, and were dispersed over the upper two-thirds of both lungs. A diffuse infiltration was noted in the region of the right pulmonary hilus. The clinical diagnosis indicated sarcoidosis, pneumoconiosis or malignancy

Lung biopsy *(2 November 1962, B 4504/62)* Dispersed epithelioid granulomas w th admixtur of numerous multinucleated giant cells, mostly of the Langhans type. Fine reticulin fibres were found in the granulomas. Neither areas of caseation, nor acid-fast mycobacteria were observed in the histological sections.

Group II

Patients, w th clinical diagnosis of sarcoidosis, in whom lung biopsy demonstrated pulmonary tuberculosis.

Case 1

DVOR M. born *1921 female* housewife. Pulmonary lesions were detected in 1955. The tuberculi reaction was epeatedly negati e, and t no time were tuberculous mycobacteria demonstrated. Antituberculous drugs were *ineffective. After* prednisone therapy regression of the pulmonary process was bserved I 1959 a relapse occurred (Fig 3) Th tuberculin test was positi

Lung biopsy (14 March 1959 B 1205 59) *Epithelioid nodules, showing* in some places slight signs of fibrous transformation. \ areas of casea ion or calcification were found *I* some giant multinucleated cells cuoles were seen *(Fig 3 b) Simila* hanges are observed in tuberculous treated w th antituberculous drugs, in particula with INH. This pplied also a the present case, *subsequently* tuberculous mycobacteria from laryngeal sw bs wer isola ed.

Case 2

MUS. B born *1922* female factory worker *This* patient had hila adenopathy nd dispersed nodules in both lungs. The lesions were disclosed in

1957. The clinical diagnosis of sarcoidosis was established. After cortisone therapy and antituberculous drugs the pulmonary process improved, but in 1959 relapse occurred.

Lung biopsy (26 March, 1959. B 1322/59): Fibrocaseous lesion with dispersed miliary nodules in the surrounding collapsed lung parenchyma. The pattern was suggestive of tuberculous etiology.

Case 3

HOM. O born 1934, construction worker. Dispersed infiltrative pulmonary lesions with enlarged bronchopulmonary and paratracheal lymph nodes (Fig. 4) were revealed by X-ray examination in November 1958. We considered the disease to be non-specific dispersed bronchopneumonia, later Hodgkin disease or sarcoidosis.

Lung biopsy (3 March, 1959, B 1039/59): Numerous epithelioid miliary nodules without signs of caseation, showing in some places narrow lymphocytic borders. In two places tiny intraplasmatic calcinosiderotic inclusion bodies were found in giant multinucleated cells (Fig. 4 b). The histological pattern narrowed down the diagnosis to such an extent that we decided to combine antituberculous drugs with prednisone. One year after the onset of the symptoms, the pulmonary process was healed ad integrum.

Group III.

In these patients difficulties were encountered in the differential diagnosis of occupational pneumopathies, tuberculosis and sarcoidosis.

Case 1

MUT. R., born 1914, waiter. During the war he worked in a quarry and after the war as glass cutter for short time. The lung lesions were revealed in 1947. In 1954 the diagnosis of pneumoconiosis was established. In 1957 the diagnosis was changed to sarcoidosis and the patient was treated with Amphen and cortisone, but without effect.

Fig. 5 shows the chest X-ray film from 13 September 1959, when lung biopsy was performed. Histological studies (B 3994/59) showed several epithelioid nodules, fusing in some places, and with isolated findings of centrally situated caseation (Fig. 5 b) indicating the tuberculous character of the lesions.

Case 2

PEL. A., born 1905, glass blower. For 15 years he was engaged in the production of neon tubes. He came in contact with luminescent powder containing 4 per cent of beryllium. His chest film from 1959 showed mixed pattern of reticular and nodular densities in the central parts of both lungs.

Lung biopsy (15 December 1959, B 5355/59): Follicular and unusual focal accumulation of lymphadenoid tissue. Characteristic beryllium-granulomas were not found and spectral analysis of the incinerated tissue failed to demonstrate beryllium, but an X-ray diffractogram of lung tissue specimen was almost analogous to the diffractogram of the powder containing beryllium, and chemical microdetermination according to Sulcek revealed also beryllium in the lung tissue. In spite of these findings diagnosis of berylliosis was established.

Summary

On the basis of our experience, although this is not very extensive, we believe that lung biopsy is very valuable method for the detection of pulmonary sarcoidosis, especially for its miliary nodular and reticulonodular forms.

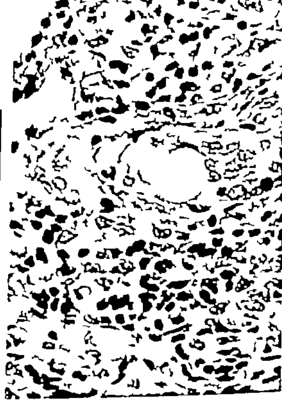
This method could be of special interest also for the detection of bronchiolar sarcoidosis described by Turiaf and Uehlinger. According to Uehlinger the histological pattern of this form of sarcoidosis is characterized by numerous non-caseous subepithelial granulomas found in the mucous membrane of the smaller bronchi and bronchioles, particularly at the point where the respiratory bronchioles open into the alveoli.

The resulting bronchiolostenosis leads to respiratory insufficiency of the ventilatory type. Since this particular form of sarcoidosis cannot be detected by X-ray examination, lung biopsy is valuable method which affords the possibility of elucidating the underlying cause of severe dyspnea in these cases.

In patients previously treated with antituberculous drugs, histological differentiation between pulmonary sarcoidosis and tuberculosis in lung specimen sometimes presents difficulties. We consider it an important fact, that lung biopsy represents today a safe and cosmetically harmless method, so that in future it may be used on larger scale also in the diagnosis of the initial stages of pulmonary sarcoidosis.



Fig 4 a. HOM. O born 1934



b. Mi-fo, 4542, 600 x.



Fig. 5. a. MUT S., born 1914.



b. Mi-fo 4540, 135 x.

Sarkoidose angenommen. Dabei konnte 88mal, also in 55,7 %, der klinische Befund einer Sarkoidose durch das histologische Ergebnis gesichert werden. Wir sind also zu ähnlichen Resultaten gelangt wie Hedvall mit etwa 50 bis 60 % und wie Wurm mit etwa 60 %.

Für diejenigen Fälle, in denen die Methode versagte, muß man wohl die vielerlei Verschiedenheiten in Rechnung stellen, die sich bei der Ausbreitung des Krankheitsprozesses ergeben können. Gebel hat außerdem die Ansicht geäußert, daß eine Blockierung und Verödung der Lymphbahnen nach vorausgegangenen bronchopulmonalen Infekten die Ausbreitung reaktiver Veränderungen verhindern kann. Wir selbst sind auch davon überzeugt, daß durch eine Verfeinerung der histologischen Technik sich die Ausbeute verbessern ließe. Daß man auch auf minutiöse Befunde gefaßt sein muß, zeigt eine Mitteilung von Schwarz und Wilhelm, die zwei Mal typische Veränderungen zwischen den einzelnen Fettläppchen gefunden haben.

Komplikationen, über die u. a. Hedvall berichtet hat, sind bei unseren Patienten nicht beobachtet worden.

Ein besonderes Problem bietet die Deutung und Bewertung der erhobenen histologischen Befunde. Es ist hinreichend bekannt, daß neben relativ eindeutigen Ergebnissen auch solche zweifelhafter Natur registriert werden. So fanden sich auch unter unserem Material 3mal Epitheloidzellknötchen mit ausgesprochenen Verkäsungen, obwohl das klinische Bild für eine Sarkoidose sprach.

Mylius und Schürmann haben in ihrer klassischen Arbeit auf das Nebeneinander verkäsender und granulomatöser Veränderungen in einem Organismus hingewiesen und damit schon vor 30 Jahren auf die gelegentlich bei der Grenzziehung zwischen Sarkoidose und Tuberkulose auftretenden Schwierigkeiten aufmerksam gemacht. Die Klinik hat uns seitdem immer wieder Beweise für unentschiedene Reaktionslagen, also für sogenannte Zwischenformen, geliefert (Lindig), die sich natürlich auch in den Ergebnissen der Scalenusbiopsie widerspiegeln müssen.

Nach unserer Auffassung sind daher die Resultate der Scalenusbiopsie nur in den Händen des Klinikers verwertbar, dann aber eine nicht zu entbehrende Stütze für die Diagnose einer Sarkoidose.

Aus der Robert Koch Klinik, Leipzig, Deutsche Demokratische Republik

Erfahrungen mit der Scalene Node Biopsy

WALTER LINDIG

Die bei jedem Verdacht auf Sarkoidose auftauchenden differentialdiagnostischen, prognostischen und therapeutischen Probleme drängen auf eine weitgehende Sicherung der Diagnose. Man war daher immer bestrebt, die Beschaffenheit der pathologischen Gewebsstruktur zur Klärung heranzuziehen. Aber erst die von *Daniels 1949* angegebene scalen node biopsy gab die Möglichkeit, geeignetes Lymphknotenmaterial routinemäßig zu gewinnen und die Diagnose auch im morphologischen Bereich zu untermauern.

Aus Schweden hat 1958 Hedvall ausführlich über die mit dieser Methode erzielten Ergebnisse berichtet. Im deutschen Schrifttum finden sich Mitteilungen von Gebel, Habicht, Kovacs sowie von Norvat und *Di Biasi*. Forschbach gab darüber hinaus 1962 eine Übersicht aus 31 Veröffentlichungen mit rund 3300 Fällen und stellte fest, daß die mit der Scalenusbiopsie erzielten positiven Resultate zwischen 13 und 100 schwanken. Er macht für diese Unterschiede nicht die Leistungsfähigkeit der Methode, sondern äußere Umstände verantwortlich, wie unterschiedliche Operationstechnik, Wahl der falschen Seite und die Verschiedenheiten in der Indikationsstellung.

Es soll hier nicht über die Frage diskutiert werden, wo der primäre Sitz der Erkrankung zu suchen ist. Es wird jedoch heute kaum bezweifelt, daß die lymphonodi bronchotracheales in der ersten Krankheitsphase befallen werden. Von da gelangt das auslösende Agens bei weiterer Ausbreitung des Prozesses über die trunci bronchomediastinales auch zu den lymphonodi supraclaviculares, die nach Töndury eine Verbindung der verschiedenen Lymphgefäßsysteme des inneren und äußeren Brustbereichs herstellen.

Wir selbst haben die Scalenusbiopsie seit 1958 zur Klärung verschiedener differentialdiagnostischer Fragen herangezogen, routinemäßig bei der Sarkoidose seit *1959*. Meine Mitarbeiter Häntzsch und Schröder haben zusammen mit Gerth (Pathologisches Institut am Bezirkskrankenhaus St. Georg in Leipzig) die Ergebnisse der bei unseren Patienten aus verschiedenen Gründen vorgenommenen Scalenusbiopsien zusammengestellt. Es fanden sich insgesamt 257 verwertbare Fälle, darunter *151* Männer und *106* Frauen. Operiert wurde fast immer rechts. Nur wenn nach dem Röntgenbefund ein überwiegender Befall der linken Lungensegment 1 bis 3 anzunehmen war, wurde die linke Seite gewählt.

224mal wurden mit dem Fettgewebe Lymphknoten entfernt, also in *87,1*. 33mal, also in *12,9* fand sich bei der Untersuchung kein lymphatisches Gewebe. Der Eingriff wurde von einem sehr erfahrenen Chirurgen durchgeführt, dem die speziellen Fragestellungen auf Grund enger Zusammenarbeit mit unserer Klinik geläufig waren. Es besteht daher Gewähr, daß alle Möglichkeiten ausgeschöpft und sowohl die lymphonodi supraclaviculares laterales als auch erreichbare Lymphknoten aus dem oberen Mediastinalgebiet mit entfernt worden sind.

158mal, also in *61,4* des Gesamtmaterials, wurde die Scalenusbiopsie wegen einer

Sarkoidose angenommen. Dabei konnte 8mal, also in 33,7 der klinische Befund einer Sarkoidose durch das histologische Ergebnis gesichert werden. Wir sind also zu ähnlichen Resultaten gelangt wie Hedvall mit etwa 50 bis 60 und wie Wurm mit etwa 60 .

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Scalene Lymph Node Biopsy in Cases of Erythema Nodosum

T G Milliken and P E. R. Tattersall

This paper describes the results of scalene lymph node biopsy in an unselected series of 43 cases of erythema nodosum, and of Kveim tests on a further series of 24 cases from the same population

Kerley (1) first noted the association of bilateral hilar adenopathy with erythema nodosum in 12 young adults, 8 of whom developed sarcoid lung infiltrations. He suggested that erythema nodosum with bilateral hilar adenopathy and negative tuberculin tests was most likely due to sarcoidosis while unilateral hilar adenopathy and tuberculin sensitivity pointed to a tuberculous origin. Lofgren (2) obtained histological proof of sarcoidosis in 25 of 113 cases of erythema nodosum.

James, *et al* (3) found histological evidence of sarcoidosis or positive Kveim tests in all of 27 cases of erythema nodosum, 24 of whom had bilateral hilar adenopathy and 3 unilateral (paratracheal) adenopathy These appear to have been selected on the basis of the finding of histological proof of sarcoidosis. Later James (4) found histological evidence of sarcoidosis or positive Kveim tests in 74 of 170 cases of erythema nodosum. In addition, he ascribed the aetiology in a proportion of the remainder to streptococcal infection because of raised serum antistreptolysin titres.

Methods

In 1957 Kveim antigen was difficult to obtain and there was then perhaps still some doubt as to its specificity Cases of erythema nodosum had been occurring fairly frequently in our hospital practice, and in view of the above reports of the finding of sarcoidosis in such a high proportion we decided to investigate a consecutive and as far as possible unselected series by scalene lymph node biopsy in addition to the more usual investigations. This was considered might give a faithful reflection of any lung pathology

All doctors in the area were therefore invited to send all their cases of erythema nodosum to the two hospitals, which serve a population of 134 000 in a rural area of some 2000 square miles in Northern Ireland. Scalene node biopsy was offered to all patients on a voluntary basis, with an explanation that it was largely a research procedure to try to find the cause of the illness. Only eight cases refused permission. Not all patients with erythema nodosum were willing to come to hospital for investigation, but at least the cases investigated were unselected from clinical or radiological aspects, apart from the presence of erythema nodosum.

Right sided scalene lymph node biopsy was carried out in each case, and if no lymphatic tissue was obvious the pre-scalene pad of fat was resected and serially sectioned at multiple levels.

Results

Scalene Node Biopsy

In the five years 1958—1963, 47 patients had scalene lymph node biopsy 36 female and 11

Table I. Erythema Nodosum 45 Unselected Cases: Scalene Node Biopsy related to Chest Radiograph findings

Chest Radiograph	Scalene Node Sarcoid	Scalene Node Negative
Bilateral Hilar Adenopathy	18	2
Unilateral Hilar Adenopathy	5	2
Normal	3	13
Total	26 = 60	17 = 40

Table II. Erythema Nodosum 24 Unselected Cases: K. eim Tests related to Chest Radiograph findings

Chest Radiograph	K. eim Positive	K. eim Negative
Bilateral Hilar Adenopathy	9	0
Unilateral Hilar Adenopathy	2	1
Normal	5	7
Total	16 = 66.6 %	8 = 33.3

male. Their ages ranged from 17 to 78 years. Table I shows the results of scalene node biopsy correlated with chest radiograph findings.

It will be seen that the highest proportion of sarcoid scalene nodes occurs in the group showing bilateral hilar adenopathy i.e. 18 out of 20. However no fewer than 5 out of 7 cases with unilateral hilar adenopathy showed sarcoid tissue, and also 3 out of 16 cases with normal chest radiographs. This shows that erythema nodosum may be associated with sarcoid aetiology whether the chest radiograph reveals bilateral or unilateral hilar adenopathy or indeed appears normal.

Tuberculin Sensitivity

There was no correlation between the finding of sarcoid histology and tuberculin sensitivity which was randomly distributed in all groups, the overall incidence being 60 % in scalene negative cases and 40 in sarcoidosis.

Kveim Tests

Further evidence that majority of cases of erythema nodosum in this area are associated with sarcoidosis was obtained when Kveim antigen became available in 1960. Some of the cases that refused scalene node biopsy together with further series of unselected cases, has had Kveim tests carried out. The results of these 24 K. eim tests correlated with chest radiograph findings are shown in Table II

Although the figures are small, it will be seen that the proportions in the various groups are much the same with K eim tests and scalene node biopsy. Tuberculin sensitivity did not correlate with any of the groups, again part from higher incidence of 60 in the K. eim negative cases.

Streptococcal Infection

Evidence of streptococcal infection in the form of raised antistreptolysin O titres has been found in proportion of cases of erythema nodosum. In this series of 67 cases significantly raised antistreptolysin titres (greater than 320 Todd units) were found in 8 cases. However 4 of these had sarcoid histology on scalene node biopsy so that streptococcal infection and sarcoidosis are not mutually exclusive in their association with erythema nodosum.

Discussion

The conclusion is that in the population examined 63 of cases of erythema nodosum are associated with sarcoidosis. Although the majority of cases with sarcoidosis falls in the group with bilateral hilar adenopathy and negative tuberculin sensitivity, sarcoidosis as shown by scalene lymph node histology and positive K. eim test may also be associated with unilateral hilar adenopathy normal chest radiograph, tuberculin sensitivity and high antistreptolysin O titre.

It could be argued that a scalene lymph node might lie at the periphery of a group of nodes draining a tuberculous lesion and still show sarcoid histology like a hard tubercle and this is agreed However if this were so it is unusual that *not even one node out of* the 26 showed caseation in any of the lesions. There were in fact only two types of abnormality seen *histologically in all 43* scalene nodes—non-caseating granuloma and a non-specific reaction.

Reports in the literature of investigations into the aetiology of erythema nodosum always list multiple factors such as tuberculosis, streptococcal infection, drugs, leprosy histoplasmosis, coccidioidomycosis and sarcoidosis. There is, however always a group in which no cause can be found. The search for histological proof of sarcoidosis in the present series was limited to scalene node biopsy and Kveim tests. Sarcoid tissue may of course be obtained from many other biopsy sites. Also the effectiveness of the Kveim test depends on the use of a potent antigen at a responsive stage of the disease. We had two batches of antigen, and the second was significantly more potent than the first in producing positive results. It is therefore possible that a higher proportion of cases would be found to be associated with sarcoidosis by more exhaustive methods.

In this group also were many patients in whom the erythema nodosum followed a complaint of painful throat and who had no evidence of streptococcal infection. These could have been of virus origin. Of the 8 cases which had a significant rise in antistreptolysin 0 titre, 4 showed evidence of sarcoidosis. The only other factor which has proved without doubt in this area to be associated with erythema nodosum is tuberculosis. Recently Stronge and Balmer (5) described 10 cases with erythema nodosum among 23 cases of pulmonary tuberculosis in an epidemic in a primary school. The higher proportion of our series with tuberculin sensitivity in the biopsy and Kveim negative groups suggests the probability that some of the cases in these groups were associated with tuberculous infection.

Whatever the actual level there is undoubtedly a high degree of association between erythema nodosum and sarcoidosis. Furthermore this association appears to be independent of the various factors usually considered to precipitate erythema nodosum. *It is significant also that 8 patients out of 28* with normal chest radiographs showed evidence of sarcoidosis, recalling the incidental finding of sarcoid histology in biopsy (6) and autopsy (7) material. It is suggested that erythema nodosum should be used as an indicator in susceptible populations in epidemiological studies of sarcoidosis.

Summary

Evidence of sarcoidosis was found by scalene lymph node biopsy or Kveim test in 63 of *67 unselected cases of erythema nodosum.* Although the highest proportion of cases of sarcoidosis was found as expected in those showing bilateral adenopathy 7 cases had unilateral adenopathy 8 cases normal chest radiographs, and 4 cases raised antistreptolysin 0 titres. Tuberculin sensitivity occurred randomly throughout all groups.

Acknowledgements

We are indebted to Mr R. C. R. Loane, Mr T J Wilmot and Mr J A. O Reilly for carrying out the scalene node biopsies, and to Dr J E. Morison for the histology reports.

We are also indebted to the Public Health Laboratories, Colindale, London, for supplies of Kveim antigen.

References

1 Kerley P Brit. J Radiol. 15. 155. 1942
2 Lofgren, S. Acta Med Scand 145. 424 1953.
3 James, D G Thompson, A. D & Wilcox, A. Lancet II 218. 1956.
4 James D C Brit. Med. J I 853 1961
5. Stronge R F & Balmer S. V Brit. Med. J 1519 1961
6. Symmers, W S C. Amer J Pathol. 27 493 1951
7 Milliken, T O Ulster Med. J 1942

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Muscle Biopsy in Sarcoidosis

F. Muratore

During recent years several reports in the medical literature have called attention to the involvement of skeletal muscles in systematic sarcoidosis (Longcope *et al.* Bates *et al.*, Bicker *et al.*)

Muscular involvement is part of the generalized process, and accompanies all clinical manifestations of the disease also in other systems (Bates *et al.*, Powell *et al.*)

Myers and his associates have also stressed, that although involvement of muscle is fairly common, clinical manifestations in muscle are rare (Harvey). Wallace and his associates have advocated routine biopsy of muscle in cases of suspected sarcoidosis, since they found that the muscles are frequently involved. Muscle involvement in sarcoidosis can, however, be entirely *asymptomatic* (Sundelin), or the symptoms may vary between extremely mild and particulary severe manifestations, ranging from slight cramp to most severe pain, accompanied by stiffness (Harvey).

According to Oser and his associates, in sarcoid myopathy disability depends more on the involvement of the nervous system than on muscle atrophy or progressive muscular dystrophy. As regards myopathy in Boeck sarcoid, *reports in the literature* describe cases characterized by myositis (cellular movement around the blood vessels, and muscular sheaths), epithelioid granulomas (Krabbe, Muratore *et al.*) and fibrotic myositis (Shorrison). Sometimes the granulomatous process was found to be present in all the muscles (Sundelin, Block, etc.), whereas, at other times, it was observed *only in some*: triceps, gastrocnemius (Lickarew Nitemeyer) gastrocnemius and pectoral (Harvey).

It has been described very rarely in an isolated location in one muscle only (Ammitzboll). In nine of our cases muscular biopsy was performed (gastrocnemius) and, with the exception of one case, we have invariably succeeded in detecting a certain involvement, even if it was not always possible to demonstrate the classical sarcoid granulomas.

On a few occasions, we have found granulomas present which attacked and destroyed some muscular fibres (fig. 1 and 2). At other times we have observed, alongside of the *classical lymphoglandular process, star-shaped* granulomas, enclosing some blood-vessels with epithelioid cells (fig. 3) or classical sarcoid granulomas (fig. 4) (Muratore & Vulpis).

In one case sarcoidosis was suspected, and lymphoglandular biopsy revealed "reticuloendotheliosis" (fig. 5). A few months later muscular biopsy showed a granulomatous process, with epithelioid cells (fig. 6). After another four months, a second lymphoglandular biopsy revealed the classical sarcoid picture (fig. 7) (Muratore & Vulpis).

In some other cases lymphoglandular biopsy showed a process of aspecific lymphopathy (fig. 8), whereas muscular biopsy demonstrated a process of widespread granulomas with epithelioid cells, and a few star

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Reports in the literature of investigations into the aetiology of erythema nodosum always list multiple factors such as tuberculosis, streptococcal infection, drugs, leprosy histoplasmosis, coccidioidomycosis and sarcoidosis. There is, however always a group in which no cause can be found. The search for histological proof of sarcoidosis in the present series was limited to scalene node biopsy and Kveim tests. Sarcoid tissue may of course be obtained from many other biopsy sites. Also, the effectiveness of the Kveim test depends on the use of a potent antigen at a responsive stage of the disease. We had two batches of antigen, and the second was significantly more potent than the first in producing positive results. It is therefore possible that a higher proportion of cases would be found to be associated with sarcoidosis by more exhaustive methods.

In this group also were many patients in whom the erythema nodosum followed a complaint of painful throat and who had no evidence of streptococcal infection. These could have been of virus origin. Of the 8 cases which had a significant rise in antistreptolysin 0 titre, 4 showed evidence of sarcoidosis. The only other factor which has proved without doubt in this area to be associated with erythema nodosum is tuberculosis. Recently Stronge and Balmer (5) described 10 cases with erythema nodosum among 93 cases of pulmonary tuberculosis in an epidemic in a primary school. The higher proportion of our series with tuberculin sensitivity in the biopsy and Kveim negative groups suggests the probability that some of the cases in these groups were associated with tuberculous infection.

Whatever the actual level, there is undoubtedly a high degree of association between erythema nodosum and sarcoidosis. Furthermore, this association appears to be independent of the various factors usually considered to precipitate erythema nodosum. It is significant also that 8 patients out of 28 with normal chest radiographs showed evidence of sarcoidosis, recalling the incidental finding of sarcoid histology in biopsy (6) and autopsy (7) material. It is suggested that erythema nodosum should be used as an indicator in susceptible populations in epidemiological studies of sarcoidosis.

Summary

Evidence of sarcoidosis was found by scalene lymph node biopsy or Kveim test in 63 % of 67 unselected cases of erythema nodosum. Although the highest proportion of cases of sarcoidosis was found as expected in those showing bilateral adenopathy 7 cases had unilateral adenopathy 8 cases normal chest radiographs, and 4 cases raised antistreptolysin 0 titres. Tuberculin sensitivity occurred randomly throughout all groups.

Acknowledgements

We are indebted to Mr R. C. R. Loane, Mr T J Wilmot and Mr J A. O'Reilly for carrying out the scalene node biopsies, and to Dr J E. Morison for the histology reports.

We are also indebted to the Public Health Laboratories, Colindale, London, for supplies of Kveim antigen.

References

1 Kerley P Brit. J Radiol. 15 155, 1942.
2 Lofgren, S. Acta Med. Scand. 145 424 1953.
3 James. D. G Thompson, A. D & Willcox, A. Lancet II 218, 1956.
4 James. D G Brit. Med. J I 853, 1961
5 Stronge, R. F & Balmer S. V Brit. Med. J 2. 1319, 1961
6. Symmers, W St. C. Amer J Pathol. 27 493 1951
7 Milliken, T G. Ulster Med. J 1942

Dr L[illegible]: It is of primary importance to differentiate, both clinically and anatomically between sarcoidosis of the larger bronchi and sarcoidosis of the bronchioles. The salient feature regarding the realization of the former condition lies in its diagnostic value, as only the larger bronchi may be bronchoscopically visualized. It has, however no functional consequences. Conversely sarcoidosis of the smaller bronchioles causes major functional difficulties, yet yields only minor diagnostic traces. Sarcoidosis of the respiratory bronchioles causes bronchiolostenosis with accompanying bronchial rigidity and obstructive emphysema with alveolar hypoventilation. (Fig. 1—2) This is clinically expressed by severe dyspnoea with only minimal concomitant roentgenological findings as was shown by Turiaf and Brun.

Figures demonstrating bronchiolo-sarcoidosis in regression with centrolobular obstructive emphysema



Fig. 1 General survey 4:1

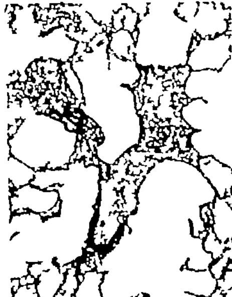


Fig. 2 Detail 40:1 L. Emma, 53 years. SN 2141/62

Dr Scadding: I agree strongly with Dr Israel about the desirability of a careful search for palpable superficial lymph-nodes suitable for biopsy before proceeding to ask for a scalene fat pad exploration. Since operations on the phrenic nerve passed out of current practice, surgeons no longer have the day-to-day familiarity with the anatomy of this region which they used to have and unfortunate incidents sometimes occur. In one of my patients, scalene node biopsy was complicated by air embolism, which the patient fortunately survived, but with some residual hemiparesis.

One other comment in relation to the subjects we have been discussing this morning. We should distinguish clearly between those procedures which are required and justified to gain new knowledge, and those which we need for the routine management of patients. Our knowledge has now advanced to such a stage that there are clinical syndromes of sarcoidosis so confidently recognizable that for practical purposes there is no need for biopsy.

Dr Ståhle: I think that all chest physicians like me have had the same experience the last years that cases with diffuse pulmonary infiltrations are increasing. *It is mostly very difficult in these cases* to get a definitive diagnosis. I have therefore asked my thoracic surgeon, Dr Janson, to do lung biopsies in these cases. In the summer 1962,


Fig. 1


Fig. 2


Fig. 3


Fig. 4

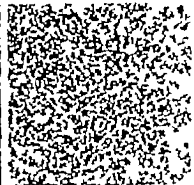
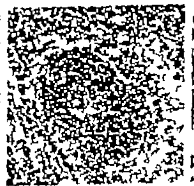
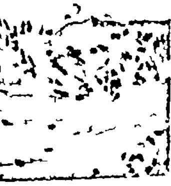

Fig. 5


Fig. 6


Fig. 7


Fig. 8


Fig. 9

shaped, giant cells (*fig. 9*) (Muratore & Vulpis). Since sarcoid granulomas are very frequently revealed by muscular biopsy we think it is advisable to insist on the introduction of this method in the diagnosis of sarcoidosis (Wallace and associates). This applies particularly to cases where lymphoglandular biopsy is uncertain, when there are no lymph nodes to remove, and although none of the other investigations have given positive results, there are well-founded clinical reasons for suspecting the presence of sarcoidosis.

microscopic lesions in the lungs before we see them on the radiogram. I suspect that there are.

Dr Stable. The case with the so-called cannon balls, I have seen in 1949. Then there were only small rounded balls. The patient consulted me again about 2 years ago; the lesions had then increased in number and size. A scalene node biopsy gave the diagnosis sarcoidosis. Thorough examinations with X-ray of the digestive channel and the kidneys have not shown any malignancy.

Dr Israel: The occurrence in sarcoidosis of large rounded shadows resembling masses, pulmonary metastases was described some years ago by Felson at Cincinnati. Lesions of this type are not uncommon and may clear completely.

Dr Lofgren Dr Berath asked if anyone had observed microscopic sarcoid lesions in the lung parenchyma of patients whose X-ray examination disclosed hilar adenopathy alone. I can answer that question. When, fifteen years ago, we started to perform bronchoscopies and bronchial biopsies in sarcoidosis it sometimes happened that small portions of pulmonary parenchyma were removed together with specimens of the bronchial wall. At least in one such case, with hilar adenopathy alone on the X-ray films, the biopsy disclosed sarcoid lesions in the parenchyma. In our papers from 1952—53, 23 cases with bronchial biopsy were reported, and the positive biopsy mentioned above was reproduced in the publication (Acta Med. Scand. Vol. 145, pp. 424—474, fig. 16).

Dr James. I would once again like to emphasize the non-specificity of some blind biopsies. It may be difficult to find the miliary granuloma in liver biopsy and pathologists may need to cut sections through the block. When he does find one of these miliary hepatic granulomas, he should only say that it is consistent with sarcoidosis, but also consistent with tuberculosis, brucellosis, many infections and even BCG vaccination. I think that the same can well apply to muscle biopsy or in any other biopsies in which only very small portion is presented for histology. I should like to know whether the granulomas in muscle biopsy are also observed in these other conditions such as tuberculosis and brucellosis.

Dr Carlens. I agree with Dr Schulbach that simple diagnostic procedures have to be tried first. There are, however, some differences of opinion what is simple and not. Prescalene node biopsy has, as Dr Israel drew attention to, given rise to complications in some patients and mediastinoscopy is considered to be an elaborate procedure mainly because it requires general anaesthesia which is not necessary in bronchoscopy. This difference will probably soon be abolished because more and more bronchoscopies are done in general anaesthesia. Then bronchoscopy and mediastinoscopy can, as we often do, be performed at the same sitting. A safe procedure of high diagnostic significance in sarcoidosis.

Dr Nourdin. If the scalene fat pad biopsy is connected with complications as we have heard from Dr Israel and mediastinoscopy gives very high rate of positive results there appears to be room for another technique. Radner (Acta Med. Scand. 152: 413, 1955) advocated the exploration of the paratracheal nodes through a small suprasternal incision.

Attractive as it appears, this technique has not been much used. In general obtained biopsy material almost throughout by the scalene node biopsy but if this technique may represent death risk even, I feel that one ought to try Radner's method which appears safer.

Dr Järvi. I agree with Dr James that one must be very careful in the interpretation of biopsies, particularly bronchial biopsies. We are doing in Finland quite many lobectomies in tuberculosis and the surgeons take specimens for histological examination from the bronchial tree both before the operation and from the removed lobe. I have examined hundreds of these specimens and very seldom I found necrosis but nearly always epithelioid tubercles, particularly if these cases have been treated with modern drugs. Therefore I would stress that if one finds only epithelioid cell granulomas, this does not necessarily mean sarcoidosis but it can be tuberculosis as well or even something else.

he visited Dr Maxwell Chamberlain in New York, and there he learned a new technic to do these biopsies which as far as I know not hitherto is more commonly known. In general anesthesia a horizontal incision, about 20 cm is done parasternally over the cartilage of the 2nd or 3rd rib generally on the right side, and then the pleura is incised. One gets a very good view of a big part of the lung and can obtain a good biopsy specimen t is also possible to take lymph nodes from the hilum. The operation can be performed even in patients w th rather bad lung function.

Since then we have made 21 such biopsies and have got a more or less definitive diagnosis in ll cases. We have found 5 cases of sarcoidosis, 5 cases of pulmonary interstitial fibrosis, type Hamman-Rich and still 2 which probably are such ones. Other diagnosis arrived t were essential pulmonary emphysema bronchiolar carcinoma, and silicosis.

For my part I prefer a lung biopsy in this way to a scalene node biopsy or a mediastinoscopy: one gets a better and surer diagnosis without increasing the operation risks very much.

Dr Siltzbach: May I first address myself to Dr Carlens In all our 200 or so bronchoscopic biopsies in patients with sarcoidosis, we fortunately have never encountered a single instance of serious bleeding W do our bronchoscopic examinations under local anesthesia but I am aware that at many bronchoscopic clinics, general anesthesia is uniformly used so that the ad antage of bronchoscopy over mediastinoscopy in this regard does not exist. I must say that I am quite surprised at the tress that some of the speakers are placing on th risks attending scalene fat-pad biopsy W have had no significant complications in our large experience w th this technique nd I would doubt that many surgeons around the world are encountering a sizeable number of complications else the operation would long before now have fallen into disfa or I certainly do not understand how one can regard an open limited lung biopsy as blander procedure than a scalene fat pad biopsy I can confirm the general usefulness of limited open lung biopsy but I rarely find it necessary to employ i among sarcoidosis patients. It seems to me that we re missing the central point about biopsy confirmation in the early diagnosis of sarcoidosis. I stress early diagnosis for of what use is t to state that one may make the diagnosis of sarcoidosis with relative ease by doing a biopsy of the cutaneous sarcoid? In the first place most patient do not exhibit cutaneous sarcoids and, when they do, the sarcoids occur almost always in the chronic phase of the illness two or more years after the onset. Since w re increasingly concerned with the early diagnosis of sarcoidosis in mass survey cases, are w to wait until the cutaneous sarcoid develops after two or more years before making the diagnosis Obviously not!

It is axiomatic that one should do the simplest and most rewarding procedure first, and in our clinics, every patient w th suspected sarcoidosis receives a Kveim test. If further biopsy confirmation is desirable, we generally go to the scalene fat-pad biopsy When pulmonary infiltrations are present with or without visible mediastinal lymph adenopathy we do four or five random bronchoscopic biopsies as I have indicated. Lately w ha e also been performing single random palate biopsies, as you heard. Gross peripheral lymph node enlargement occurs uncommonly in our series of patients and when the nodal enlargement ppears, it manifests itself considerably later than the hilar node enlargement. We consider the presence of substantially enlarged peripheral nodes as evidence of late subacute rather than early subacute sarcoidosis. Only after all of these diagnostic measures fail do we employ open lung biopsy Nor do we experience many complications with open lung biopsy But w do hold this procedure one t be undertaken after other less drastic pproaches have been exhausted. W are only now beginning to do mediastinoscopy and we are also becoming interested in Dr J M. Chamberlain s direct mediastinal approach through a small costectomy incision.

Dr Villar After analyzing 44 biopsies done on 33 cases of sarcoidosis in Portugal, I prefer the lymph-node and liver biopsies as, in my small series these gave the highest percentage of positi e results. In my experience bronchial biopsies were only positive in those cases in which macroscopic changes of the bronchial mucosa were seen. I am ery interested in trying Dr Siltzbach technique of multiple random bronchial biopsies.

Dr Birath During the last years in the University Lung Clinic in Gothenburg about 300 mediastinoscopies ha e been performed. Out of them 33 have been made for diagnostic purpose in cases suspected of sarcoidosis. All of them have been positive. Further it has been done in fiv cases where w had no diagnoses. As sarcoidosis possibly could ha been the diagnosis, these cases perhaps ought to be included i the material. They were however ll negati

I should like to ask couple of questions Dr Stähle showed very interesting radiogram of case with round lesions scattered in both lungs. I have not seen such radiogram in cases of proved sarcoidosis nd I would like ery much t hear if anybody else has seen t. Also, I would like to know from Dr Stähle how his case was confirmed having sarcoidosis. I tuberculous such lesions are described and called cannon-ball' lesions Could it ha e been such a case?

The next question is if anybody has made lung biopsy in a case of adenopathy alone, because t should be of great interest to know if there are

cours des sarcoïdoses survenant chez des malades anciennement primo-infectés et devenus tuberculino-insensibles, comme il l'est chez des individus devenus spontanément hypo-allergiques et n ayant pas de sarcoïdose. Le test au B.C.G. n a aucune aleur étiopathogénique et les épreuves pratiquées ont bien montré qu il y a ait de grandes variations dans les resultats, comme on devait y attendre. Danbolt en 1948   montré que le test d'Løvedt et Aanonsen était rarement positif chez les sujets atteints de sarcoïdose et ayant des éactions tuberculiniques négatives. Forgas, Mac Donald et Skelton ont injecté du B.C.G chez 10 malades atteints de sarcoïdose 6 d'entre eux valent des réactions tuberculiniques négatives   l'injection intra-dermique de B.C.G   provoqué une éactivation de l'allergie chez seulement 2 de ces malades qui ont eu alors une éaction de Mantoux positive. Pour les 4 malades restants ayant gardé au cours de leur sarcoïdose une intra-dermo positive, l injection de B.C.G.   pas augmenté les éactions dans l cas même la réaction est devenue secondairement égative. Il faut noter aussi que les lésions locales après injection de B.C.G ont été soit immédiates, soit retardées   elles ont même été retardées chez 2 sujets ayant cependant des éactions tuberculiniques initialement positives. Cette expérimentation sur le vivant met parfaitemen en évidence les variations et les discordances de résultats, liées d'une part à la sensibilité de la peau d'un chacun, et d'autr part à la neutralisation plus ou moins grande, suivant les sujets, des réactions de sensibilisation   la tuberculose provoquées par la maladie sarcoïdosique.

R Lemming aurait obtenu une positivité des réactions de Mantoux après vaccination au B C.G chez 30   des sujets ayant une sarcoïdose. Brocard et ses collaborateurs, en 1950, ont montré que l'injection de B.C.G vivant chez des malades atteints de sarcoïdose provoquait dans 46   des cas une réaction tuberculinique qui était éphémèr   et ne durait guère au delà de six mois, et cela sans doute par stimulation de l'allergie chez d'anciens primo-infectés. Pour notre part, nous vons pratiqué des tests au B.C.G et nous n   ons jamais obtenu soit de stimulation tuberculinique avérée soit de éaction locale particulière et précoce chez des sujets n yant jamais été primo-infectés.

Certains auteurs ont pensé que la éaction au B.C.G des malades atteints de sarcoïdose était différente de celle des sujets sains négatifs à la tuberculine. Rolf Lemming en particulier en 1942,   rapporté une observation de développement de nodules sarcoïdosiques de Boeck aux lieu et place d une accination au B.C.G. chez un homme   yant une maladie de B.B.S. tout à fait typique. La documentation iconographique emporte l'entière conviction. Ultérieurement, il est revenu sur ces faits d'après une expérimentation plus large et l'aspect sarcoïdosique des lésions se   errait surtout dans les cas demeurant anergiques et en lesquels il y aurait peu de bacilles d B.C.G. P Forgas et ses collaborateurs ont fait des prélèvements   histologiques   six   semaines près la   accination chez des sujets atteints de sarcoïdose et ils n ont pas pu retrouver de lésions de type bien caractéristique. Ils ont noté aussi que les souches de bacilles B.C.G. n étaient pas modifiées par la maladie sarcoïdosique et étaient retrouvées six semaines après au sein des prélèvements. Là encore, comme on le voit, il y a des variations et rien n est constant ou spécifique.

III Maladie Sarcoïdosique Consécutive à la Vaccination au B.C.G.

Ces faits ont une importance étio-pathogénique. En effet, si l'on croit à la théorie rattachant la maladie à un bacille de Koch de virulence atténuée la   accination au B.C.G. paraît être capable de déclencher des syndromes du même type.

Les lésions locales provoquées par la accination ont été étudiées depuis longtemps et très écemment de façon fort détaillée et pertinente par A. Vortel en Tchécoslovaquie. Les lésions locales rencontrées chez des enfants décédés d affections différentes, sans relation avec la   accination, montrent qu il peut y avoir un tissu de granulation comparable à celui de la sarcoïdose et contenant des corps astéroïdes de Schaumann. Mais nous savons bien que ces éactions histo-

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Moderator Harold L. Israel

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J Brun J Turiaf et A. Despierres

Les relations éventuelles entre la vaccination au B C.G et la maladie sarcoïdosique ont été discutées depuis longtemps. En fait, il y a des problèmes divers qui méritent attention.

I Vaccination au B C G et Sarcoïdose

La vaccination au B.C.G au cours de la sarcoïdose a été tentée pour traiter la sarcoïdose elle-même. Avec J Turiaf nous avons bien montré qu'il ne fallait rien attendre de tels traitements, qui ont été essayés avec un plein insuccès par divers auteurs tels que Sohier et Gomer à Lyon, Israel et ses collaborateurs. Bien plus, il a été jadis publié les aggravations de la maladie ou même une tuberculose secondaire (Ehrner) survenue après vaccination au B C.G au cours d'une sarcoïdose. Il est probable que dans de tels cas il s'agissait de malades qui avaient déjà été infectés de façon latente par le bacille de Koch et pour lesquels la vaccination au B.C.G était contre indiquée. L'absence d'allergie tuberculinique éventuellement provoquée de façon secondaire par la maladie de B.B.S. ne témoigne pas d'une réelle anergie humorale et n'exclue pas des phénomènes de sensibilisation, à l'occasion de réinfection artificielle.

Par contre il est bien évident que chez un sujet n'ayant jamais été contaminé par le bacille de Koch et ayant une sarcoïdose guérie la vaccination au B.C.G peut être envisagée sans aucun risque. Nous avons d'ailleurs l'observation d'une jeune fille qui, deux ans après une sarcoïdose ganglionnaire médiastinale à 20 ans, a été vaccinée au B.C.G sans aucun incident.

II Test au B.C.G et Allergie Tuberculinique au Cours de la Sarcoïdose

Nous savons tous que la diminution ou l'extinction des réactions cutanées tuberculiniques au cours de la maladie sarcoïdosique est fréquente mais non constante. Il y a donc des variations importantes dans le mode de réaction de la peau et du derme aux sommations tuberculiniques lors de l'infection sarcoïdosique. Celle-ci, chez des sujets plus ou moins anciennement primo-infectés, va empêcher dans une plus ou moins grande mesure l'apparition des réactions cutanées à la tuberculine, sans que pour cela on doive admettre la disparition réelle de l'allergie humorale. L'épreuve du test devient négative mais l'allergie persiste. En faveur de ces faits, il y a la notion fondamentale de la réapparition d'une allergie tuberculinique cutanée ancienne lors de la guérison de la maladie, sans qu'il n'y ait eu de nouvelles contaminations tuberculeuses.

Le test au B.C.G d'Ustvedt et Aanonsen permet de déceler des allergies dites infra-tuberculiniques il peut donc être positif au

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II Test au B C G et Allergie Tuberculinique au Cours de la Sarcoïdose

Nous savons tous que la diminution ou l'extinction des réactions cutanées tuberculiniques au cours de la maladie sarcoïdosique est fréquente mais non constante. Il y a donc des variations importantes dans le mode de réaction de la peau et du derme aux sommations tuberculiniques lors de l'infection sarcoïdosique. Celle-ci, chez des sujets plus ou moins anciennement primo-infectés, va empêcher dans une plus ou moins grande mesure l apparition des réactions cutanées à la tuberculine, sans que pour cela on doive admettre la disparition réelle de l allergie humorale. L épreuve du test devient négative mais l allergie persiste En faveur de ces faits, il y a la notion fondamentale de la réapparition *d une allergie tuberculinique cutanée* ancienne lors de la guérison d la maladie, sans qu il n y ait eu de nouvelles contaminations tuberculeuses.

Le test au B.C.G d Ustvedt et Aanonsen permet de déceler des allergies dites infra-tuberculiniques il peut donc être positif au

TABLEAU II

Années universitaires étudiants 19 à 27 ans	Nbre. d'examens pulmonaires	Nbre. de cuti faites	Nbre. de cuti positives	Nbre. de dépistages tuberculose pulmonaire	cas de Besnier-Boeck Schaumann
1956—1957	8.553	5.717	3.170 (61 après B.C.G.)	47	0
1957—1958	9.340	6.170	2.722 (113 après B.C.G.)	53	0
1958—1959	10.313	6.631	3.255 (76 après B.C.G.)	49	1 B.B.S. (après B.C.G. 1 an ant)
1959—1960	11.301	7.393	4.042 (2.353 après B.C.G.)	27	1 B.B.S.
1960—1961	12.792	8.665	4 899 (3.266 après B.C.G.)	34	1 B.B.S.
1961—1962	14 281	10 157	6.015 (4.281 après B.C.G.)	44	1 B.B.S.

En faveur de cette notion, il y a aussi des faits statistiques globaux. Deux séries méritent ici d'être signalées

1) Sur une série de 125 000 vaccinations au B.C.G., de 1948 à 1962, nous n'avons eu connaissance que de trois cas de sarcoïdose ganglionnaire pour la ville de Lyon et ses environs, la vaccination ayant porté sur des sujets de 6 ans à 20 ans.

2) Nous donnons un tableau statistique comprenant uniquement des étudiants de l'Université de Lyon (II) Nous voyons, que d'une part les cas de sarcoïdose dépistés sont peu nombreux et que, d'autre part, la vaccination au B.C.G n'a entraîné aucune augmentation du nombre d'affections du type sarcoïdosique. 66 580 étudiants furent suivis durant six ans, de 1956 à 1962 1/5 environ, soit 13 000 étudiants furent vaccinés. Or on rencontra quatre sarcoïdoses chez des sujets non vaccinés et une seule chez les sujets vaccinés par le B.C.G La proportion est donc strictement la même pour ceux qui sont vaccinés et ceux qui ne le sont pas

Il apparaît bien, en définitive, que la généralisation de la pratique de la vaccination au B.C.G. n'a pas entraîné des syndromes cliniques identifiant à ceux de la maladie sarcoïdosique.

Bibliographi

Berkhauser H. Schweiz. Med. Wsch., 1937 67 1434

Brocard, H., Brincourt. J & Vannier R. Bull. et Mém. Soc. Méd. Hôp. Paris, 1953, nº 21—22 et 23, 745—752.

Brun, J & Planchu Rev Tub. 1947 tome 11 361—367

Dahl, N. Nordisk Medicin, September 17 1948, 39 1068—71

Ehmer Acta Tub. Scand., XX, fasc. 2—4 1946, 138—157

Fried, H. & Gens, H. Tuberculose arzt. 1958, 12: 5.8.

Israel, H. L. & Sones, M. Adv Tuberc. Res 11, p. 214—263 (Karger Basel/New York 1961)

Israel, H. L., Sones, M., Stern, S. C. & Aronson, J. D Amer Rev Tuberc. 1950, 62. 408.

Lofgren, S., Strom, L. & Widstrom, G. Acta Pædiatrica 1954 vol. 43, Sup. 100 160—166.

Oudet, P & Roegel, E. Le Poumon et le Cœur 1962, 18 927

Prost, P & Wacker Th. Le Poumon et le Cœur 1962, 18: 937

Lemming, R. Acta Medica Scand. 1942. Vol. CX, fasc. II—III.

Lemming, R. Acta Tuberculosea Scand 1959 suppl. 45 29—31

Schaumann, J & Seeberg, G. Acta derm. venereol., Stockh. 1948, 28, 1.6

Turiaf, J. & Brun, J. La sarcoïdose endothoracique de Besnier-Boeck Schaumann. Expansion Scientifique Française, 1955.

Vortel, V The American Review of Respiratory Diseases, 1962, 86 236.

TABLEAU I Sarcoïdose après B.C.G

Sexe	Age	Atteinte observée	délai d'apparition après B.C.G.S
Homme	14 ans	Forme ganglionnair médiastinale	6 mois
Femme	15 ans	»	2 ans
Femme	16 ans	Forme hilaire adénopathie unilatérale	4 ms 4 ans
Homme	19 ans	Forme médiastinale	5 ms
Femme	30 ans	»	6 ans
Femme	19 ans	»	6 ans
H mme	21 ans	Forme nodulo- nfiltrative	8 ans
H mme	19 ans	Forme ganglio-pulmonaire	8 ans

pathologiques du type sarcoïdosique sont l apanage de multiples affections, d essence différente, puisque aussi bien la tuberculose que la bérylliose peuvent provoquer des réactions du même type et sans caséification. Sur le plan clinique, il serait par contre fort troublant et presque convaincant de voir apparaître des atteintes sarcoïdosiques après vaccination au B C.C En 1958 H Fried et H Genz ont rapporté 12 observations de ce type, mais elles ne peuvent entraîner la moindre conviction. En eff t, c est seulement dans 2 cas que la sarcoïdose a paru survenir dans l année qui a su vi la vaccination. Pour tous les autres cas, elle est apparue plusieurs années après le B C.G et le plus souvent à l occasion d épisodes infectieux aigus ou sub-aigus intercurrents. Il nous parait donc bien discutable de suivre ces auteurs qui partent de tels faits pour conclure à une forme atténuée de tuberculose. Löfgren a rapporté 25 cas survenus après accination au B.C.G soit de façon précoce, soit de façon tardive Nous-mêmes, nous a ons observé 7 cas de sarcoïdose (5 ganglionnaires-hilaires, 1 pulmonaire et 1 ganglio-pulmonaire) chez des malades ayant été vaccinés u B.C.G L intervalle de temps a été variable entre la vaccination et l apparition de la maladie, comme le montre le tableau I

Ce qu il y a de particulier c est que chez tous nos malades la cuti était devenue secondairement négative après vaccination et avant l apparition de la maladie. Celle-ci n a pas modifié l allergie négative à la tuberculine. L évolution de l affection s est faite par la régression sous traitement cortisonique. Nous pensons donc que l apparition de cette affection a été simplement contingente et que l'on ne peut en aucun cas incriminer l'action du B.C.G de façon valable. Des faits semblables ont été rapportés à Lyon par Oudet et Roegel en avril 1962 et par Renard, Purinel et Bertrand, et les auteurs ont abouti aux mêmes conclusions. Cependant, Press et Vacker a propos d adénopathies rencontrées chez des enfants vaccinés, ont discuté les relations possibles entre des adénopathies sarcoïdosiques et la vaccination. Birkhauser en 1957 a rapporté aussi chez l'adulte 5 observations de B.B.S. pour lesquelles il discute l'influence du B C.G

A ce propos, il importe de bien séparer ce qui est d origine sarcoïdosique et ce qui ne l est pas. En 1947 l un de nous a bien montré que dans certains cas la accination au B.C.G déclenchait, dans les 4 mois qui suivaient la accination, des adénopathies médiastinales, d ailleurs sans gravité, évoluant de façon transitoire et dépistées par examen systématique Ces faits ont été confirmés par les travaux de Gernez Rieux portant sur 10 000 enfants subissant tous les mois des contrôles radio-photographiques. D autre part il ar ri e que des sujets vaccinés au B.C.G fassent secondairement une primo-infection à l occasion d une contamination bacillaire irulente survenant sur un terrain non franchement immunisé mais, dans tous ces cas l allergie tuberculinique était exacerbée

On voit donc que les observations de maladie sarcoïdosique rencontrées par nous chez d anciens vaccinés sont différentes dans leur forme ganglionnaire des adénopathies précoces du B.C.G ou des adénopathies de réinfection tuberculeuse et que la sarcoïdose a paru survenir de façon autonome et à titre fortuit.

Table I. BCG-Vaccinations in Berlin-West

1949/50	109 614	1957	1,556
1951	118	1958	2,593
1952	145	1959	3,181
1953	429	1960	9,669
1954	293	1961	14 127
1955	310	1962	17 601
1956	498		

Registered Population without Age Limit

year	total	male	female
1950	2 146 952	911 504	1 235 448
1961	2 197 408	929 005	1 268 403

ren. These age groups varied considerably in size (1941 = 16,647 females and 17,310 males; 1946 = 6,840 females and 7 190 males) The number of persons vaccinated fluctuated between 0.2 (1928) and 43.5 per cent (1942)

This was due not only to the varying degree of tuberculosis infection in the different age groups, but also to the fact that the possibilities for approaching the groups varied considerably The curve of the results of tuberculin testing among the West Berlin population showed that even in 1950, 72 per cent of the female and 78 per cent of the male subjects in the age group from 19 to 20 years were tuberculin-positive.

Out of 205 female sarcoidosis patients, 155 were born before November 1 1928 out of the 114 male patients, 34 were born before that date. It is certain that these persons had not been BCG-vaccinated because of their age for it was only at later date that isolated subjects, among persons exposed to tuberculosis at their work were BCG-vaccinated.

This definitely proves that, in our patients, BCG-vaccinations have not directly provoked sarcoidosis. If the BCG germ is to be considered an agent in this sense all, then comparison could only be made with similar latencies in leprous infections.

The question, causality or accidental coincidence, was checked in the following manner: As far as age groups of accinated and non-vaccinated persons were ailable, these are collectively arranged in 5-year groups according to sex. Beginning with the standard morbidity of non-vaccinated persons suffering from sarcoidosis, w calculated by means of the standardizing formula, according

to the method of "expected events" $\frac{\Sigma n_i\ m_i}{\Sigma n_i\ M}$

and obtained the deviation in the observed sarcoidosis cases among vaccinated persons, from the sarcoidosis cases to be expected: for the female group the figure was 117.91 % and for the male group 93.044 %. No significance can be deduced from these values. W can infer an accidental coincidence as long as more comprehensive observations, such as the use of other vaccines, or other climate conditions do not lead to the publication of contrary results obtained by incontestable methods.

Peculiarities in BCG-vaccinated Sarcoidosis Cases

The 22 female and 15 male sarcoidosis-patients — all BCG-vaccinated — were diagnosed in stage I or II (thoracic manifestation) Thus, the prognosis was mostly more favourable than on the verage for our 319 cases. Furthermore, only 8 cases showed definite extrathoracic manifestations (lymph nodes, spleen, conjunctiva) this is again less than the average for considerably older patients. One case started with erythema nodosum. Four cases healed spontaneously Otherwise the course of the disease did not greatly differ from that of the non-vaccinated persons, as recidivation and therapy resistance (2 cases for 7 and 8 years respectively) could be established also for these persons. Three of the patients have since become pregnant.

In the ascendence of 6 cases predisposition to tuberculosis was established. Three patients clearly showed tuberculous exposure for longer period of time.

BCG and Sarcoidosis in Berlin West

K. H. Fried, Berlin, Germany

As you may know all BCG-vaccinations came almost to a complete standstill in Germany owing to the tragic mishap of contamination of cultures in Luebeck in 1929/30 although tuberculosis showed an unfavourable trend in Central Europe and no possibility of effective medical treatment existed.

On account of the effects of the war there was a sharp increase of tuberculous morbidity and mortality particularly in the large cities in Germany. Apart from the well known methods of treatment (mass radiography, tuberculin testing, special clinics and therapy) all prophylactic measures, especially BCG-vaccination had to be utilized.

Thanks to the Swedish Red Cross, BCG vaccinations were carried out on a large scale in Germany during 1949/50. It was often only because of the efforts of Swedish colleagues and the use of Scandinavian vaccines that the widespread scepticism of the population could be overcome.

In Berlin, where—compared with the rest of Germany—the situation with regard to tuberculosis was most unfavourable, numerous small children and school children up to 1 years of age were BCG-vaccinated during 1949/50. In the following years, mainly new-born babies or infants were vaccinated who came from a tuberculous environment.

Because of the special selection and the small number of vaccinated persons initially available, all vaccinations *after 1950* are excluded in the following report.

In 1958, Genz and Fried reported on the first 12 sarcoidosis cases among BCG-vaccinated persons in Berlin. The shortest interval between vaccination and sarcoidosis manifestation was 18 months, in other cases it was more than 3 years. Thus, these cases can be distinguished from isolated ones, where,—as reported—sarcoidosis occurred already after one to nine months. More recent literature has not especially dealt with the problem of sarcoidosis and BCG e.g. the 25 or more cases selected from 212 patients, which were observed by Lofgren and Lundbäck. Most studies in this field lack comparative value with reference to the age structure of the population, the number of non-vaccinated persons, and the occurrence of tuberculosis among vaccinated and non-vaccinated patients. Thus, it was not possible to casuistically establish, whether an accidental coincidence or a causal connection existed. Birkhauser, Genz and Fried, as well as Ganguin and other investigators were inclined to accept the latter view but they had no proof, owing to the small number of cases. Therefore, I am pleased to follow Löfgren's suggestion, namely to study the question once again on the basis of material available in Berlin.

As no differentiated data were available, 4000 BCG index-cards were selected at random and from these the various numbers of vaccinated persons and their age groups were determined.

Lack of space does not permit the inclusion of comprehensive statistics.

The vaccination was offered to persons belonging to the age groups between 1928 and 1950, especially to infants and school child

mon tuberculosis, but may possibly favour that of the more "benign" sarcoidosis. This view is supported by our frequent observations and by those of others investigators regarding so-called transitional cases; the demonstration of tuberculous tissue, which is no longer rare the frequent demonstration of tuberculous calcified foci the repeated occurrence of sarcoidosis in the same families, and the positive tuberculin reaction before, during or after sarcoidosis. Former BCG practice is not invalidated by these observations.

The therapeutic BCG-vaccination of sarcoidosis patients has been carried out only 4 times and with varying results therefore, no definite statement can be made concerning that.

References

Birkhäuser H. Schweiz. med. Wschr 1957 1434.

Ellmann, Ph. Brit. med. J 1939, 1433.

Forgacs, P McDonald, C. K. & Skelton, M. O., Lancet 1957 I 188.

Freerksen, E. Tbk.arzt 1957 65.

Fried, K. H. & Genz, H. Tbk.arzt 1958, 534.

Ganguin, H. G. Allergie & Asthma 1956, 88.

Glanzmann, E. Aerztl. Mhefte 4 (1948) 621

James, D. G. Brit. med. J 1956, 900.

Larsen, A. K. Nord. Med., Stockholm 43 (1950), 170.

Löfgren, S. & Lundbäck, H. Acta med. Scand. 1952, 265.

Pfisterer R., Wenpl, H. & Herzog, H. Helvet. med. acta 21 (1954) 439.

Richards, L. D & Steingold, L. Brit. J Tbc. 46 (1952) 163.

Staubli, C. Praxis, Bern, 1953 1006.

Tornell, E. Nord. Med., Stockh. 1954, 996.

DISCUSSION

Dr. Purriel In Uruguay vaccination with BCG was started in 1927 On the relation between vaccination and sarcoidosis w found that of 40 patients under 50 years of age, only 10 had been vaccinated (20 %) I is all we can say about the relationship between sarcoidosis and vaccination.

Dr Fried Because of shortage of time, the test results *after* BCG-vaccination carried through in Berlin in the framework of the mass vaccination campaign of 1949/50 are *not complete*. A small number of vaccinated persons did not even come for Moro testing other negative reactors objected to Mantoux injection. W calculate that 85 to 90 per cent ere successfully vaccinated, although only 77 7 per cent of those vaccinated reacted *positively to the tuberculin-patch-test and 13 per* cent showed positive reactions to Mantoux (50 T U) I turned out during later examinations, e.g by general practitioners or during school health examinations that among those insufficiently tested or not tested at all there were still number of tuberculin-positive reactions.

Out of our 37 BCG-vaccinated persons who later on contracted sarcoidosis, 29 had clearly been tuberculin-positive. Five cases showed negative reaction to patch-test intracutaneous tests *ere not applied. In three cases*, the test results could not be established. However two showed BCG-vaccination marks at the typical spot, so that successful vaccination was very likely Out of the eight patients whose test results after vaccination were lacking or incomplete, four showed positive tuberculin reactions at the time of discovery of sarcoidosis. Three patients received tuberculin injections up to only 10 T U and reacted negatively Only one case showed tuberculin-negative reactions at the beginning of the disease, even after injection of 1000 T U

On the basis of these results, I think I may rule out that unsuccessfully vaccinated persons (tuberculin negative reaction) contract sarcoidosis more frequently than those, in whom BCG-vaccination had caused tuberculin sensitivity

Dr Jörgensen Ich möchte on genetischer Seite anhand von drei Familienbeobachtungen einen kleinen Beitrag zu der angeschnittenen Frage liefern.

In einem Fall erkrankten Bruder und Schwester an *Sarkoidose. Jedoch war nur der Bruder* 2 1/2 Jahre zuvor mit BCG geimpft worden (Abb. 1)

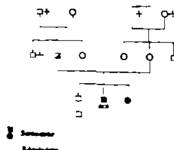


Abb 1

TABLE II Age of sarcoidosis patients (Berlin West) including BCG-vaccinated persons at the initial detection of sarcoidosis (1949—1962)

age group years	Female total	Female BCG-vaccinated	Male total	Male BCG-vaccinated	Total BCG-vaccinated cases
under 0 to 10	1	—	—	—	—
under 10 to 15	9	2	13	5	7
under 15 to 20	36	14	21	4	18
under 20 to 25	28	6	29	6	12
25 and over	131	—	51	—	—
Total	205	22	114	15	37

TABLE III Sarcoidosis cases among BCG vaccinated persons according to age groups

year of birth	female	male	year of birth	femal	male
1945	1	1	1938	2	1
1944	1	1	1937	1	—
1943	1	2	1936	1	1
1942	3	1	1935	3	1
1941	4	1	1934	—	—
1940	3	2	1933	1	1
1939	1	3			

TABLE IV Intervals between BCG-Vaccination and initial detection of sarcoidosis

	Femal	Mal
Shortest interval	3 years 1 month	1 year 6 months
Longest interval	12 » 4 months	12 years 4 »
Average interval	8 » 2 »	7 » 5 »

In this connection a striking observation can be discussed: of the 22 female patients, 18 had brothers and sisters; 7 of these, who were not BCG-vaccinated, contracted tuberculosis! Nine of the male patients had brothers and sisters; two of these, who were non-vaccinated, contracted tuberculosis. According to our epidemiological analysis—these results are much higher than can possibly be accounted for by accidental coincidence.

After many years of experience with BCG it seems incontestable that successful vaccination brings about an increased resistance to virulent tuberculous superinfection. It cannot be debated here whether it is justifiable to postulate an interference phenomenon (Freerksen).

In Berlin too, the occurrence of tuberculous diseases among vaccinated persons is ten times less than in non vaccinated persons. The disease itself is often mitigated.

Our opinion is that, in a certain type of constitution (of which so far we have no detailed conception) BCG-vaccination leads to a hyperergia, which, in the case of superinfection, prevents the development of com-

Dr. WALLGREN: I think I have an answer to give to Dr. One and Dr Israel. Last year before defending thesis on tuberculin, I had tested different tuberculin preparations on the pupils of the nursing school in Umeå. On that occasion I went through the material 10 years back to find out if there was any higher morbidity in tuberculosis or sarcoidosis in BCG-vaccinated student nurses who had failed to react to tuberculin. There were 46 non-reactors who had been vaccinated once or several times among roughly 550 nurses (8) and some later developed sarcoidosis or tuberculosis.

Dr SOMMER: *May I add only few words to the* paper of Dr Fried. I think we have to be thankful for his statistic work but I think we can never accept his conclusions saying that tuberculous infection in BCG vaccinated subjects has more frequently the tendency of converting to the clinical picture of sarcoidosis than to tuberculosis. This may be hypothesis, but it is completely unproved.

TABLE TORSELL. Prevalence *of* sarcoidosis among persons previously BCG-vaccinated and those not vaccinated, in different age groups, according to mass photofluorographic survey carried out in Boda during 1950—51
Participation 92 per cent of the inhabitants

Age groups (years)	No. of persons examined	Negative or non-significant findings		BCG-vaccinated per cent	Prevalence of sarcoidosis		Prevalence of post primary pulm. tubercul.	
		BCG-vacc.	Non-vacc.		BCG-vacc.	non-vacc.	BCG-vacc.	non-vacc.
1	2	3	4	5	6	7	8	9
Men								
15—19	1,637	1 170	377	71 6—73.5	1	—	2	2
20—24	1,833	829	833	45.1—50 0	2	—	—	15
25—29	2,233	672	1,324	30 1—33.7	2	1	3	25
30—34	2,122	331	1,632	15.6—16.8	—	4	—	42
15—34	7,827	3,002	4,166	38.3—42 0	5	5	5	84
					=0.17	=0 12*	=0 17	=2.0%
Women								
15—19	1,850	1,231	413	66.5—74 6	—	—	2	3
20—24	2,228	1 151	751	51.6—60.4	—	—	3	14
25—29	2,439	793	1,394	32.2—36 2	2	4	3	33
30—34	2,449	333	1,979	13.6—14 4	1	9	1	44
15—34	8,966	3,510	4,537	39.2—43 5	3	13	9	96
					—0 09*	=0.29*	—0.26	=2 1

In der anderen Familie waren Mutter und Tochter an Sarkoidose erkrankt. Hier war lediglich bei der Tochter eine BCG-Impfung vorgenommen worden (Abb. 2)

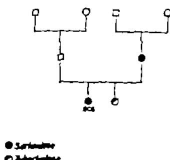


Abb. 2

Bei zwei kleinen Kindern der großen Sarkoidose-Sippe (siehe meinen Vortrag „Die Genetik der Sarkoidose") mit insgesamt 8 kranken Mitgliedern blieb die Mantouxreaktion negativ, obwohl die Kinder mit BCG schutzgeimpft waren (Abb. 3). Es liegt hier vermutlich eine genetische Determiniertheit in der Reaktion auf BCG-Bazillen vor.

Genetische Aspekte sprechen eher gegen als für einen Zusammenhang zwischen BCG-Impfung und Sarkoidose.

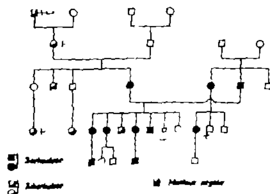


Abb. 3

Literatur

Jörgensen, G.: Sarkoidose und BCG-Impfung. Praxis Pneumol. 1964, 18,25

Dr. Törnell. On the basis of the mass photofluorography that was carried out in Borås (Sweden) during the years 1950—51 it was possible to estimate the number of persons in different age groups who had previously been BCG-vaccinated. For each age group, 400 records with negative or non-significant findings were scrutinized. The statements made in connection with the previous BCG could be verified by comparing them with the notification records which are available at the dispensary. About 8—10 % of the persons examined belonged to the group of positive findings which were not included in the figures. If it can be assumed that all these persons were not vaccinated, then the percentage in column 5 of the table (next page) will be reduced as shown by the figures on the left side of the column. It appears from the table, however that a large number of the young persons (about 40 % in the age groups, 15—34 years) were BCG-vaccinated. The subjects with sarcoidosis who were previously BCG-vaccinated (5 men and 3 women as against 5 and 13 non-vaccinated) are not more than was expected. There are therefore no reasons to suppose that BCG may cause sarcoidosis. On the contrary as seen in columns 8—9, the prevalence of post primary pulmonary tuberculosis in persons who were BCG-vaccinated, is distinctly less than expected.

Dr. Israel. Studies were carried out at Phipps Institute using a vaccine prepared by Dr. Aronson which produced conversion in 95 per cent of control subjects, only 45 per cent of patients with sarcoidosis became tuberculin-positive. No significant differences were observed between sarcoidosis patients and controls in the gross or histologic appearances at the site of BCG inoculation.

Sarcoidosis in our country, with rare exceptions, occurs in persons who have not received BCG vaccination, and we find it difficult to believe that this vaccine can be an etiologic factor in sarcoidosis. We are more interested in the possibility that failure to convert may be a manifestation of sarcoidosis. Although not generally employed in the United States, BCG vaccination has been fairly widely used in groups with special hazard of tuberculous infection such as medical students and student nurses. I have encountered many students who have failed to convert, often after repeated attempts. I recall only one who subsequently developed sarcoidosis. What is the experience of others.

I should like to conclude with the comment that on the basis of clinical, bacteriologic and epidemiologic evidence, most of us are skeptical about an etiologic relationship of tuberculosis and sarcoidosis. Nevertheless, there remain a number of observations, such as those which Dr. Fried has reported, which indicate that the controversy is not settled. I should like to cite a recently reported study which is not well known. Epstein (J.A.M.A. 180, 767, 1962) injected zirconium intradermally in 225 patients with arrested tuberculosis and 300 normal subjects. Eight subjects developed delayed granulomas at the site of injection, seven were in the tuberculosis group, suggesting that prior mycobacterial infection may contribute to sensitization resulting in granuloma formation.

indirect evidence about it may be obtainable. We all know that in Europe at present many cases of erythema nodosum are associated with sarcoidosis. Those who think that sarcoidosis is due to an unidentified specific agent would say that this is because this agent is another of those which may precipitate erythema nodosum. But it seems to me just as plausible to suppose that this association is common because in some individuals the same external agent may precipitate both erythema nodosum and sarcoidosis just as *Str. haemolyticus* may rarely precipitate both erythema nodosum and acute rheumatism.

T turn now to acute rheumatism the various manifestations of this disease—rheumatic fever rheumatic carditis, and chorea—are certainly triggered off by a haemolytic streptococcal infection, and this relationship is undoubted. Yet the haemolytic streptococcus is not discoverable in any of the specific lesions of acute rheumatism and at the time when the patient has the manifestations of this disease, the haemolytic streptococcus may not be discoverable even in the throat.

And, lastly in leprosy the difference between the tuberculoid and lepromatous types is presumably determined by differences in the immunological reactivity of the patient. Typically in the lepromatous type there ar many bacilli, and negative Mitsuda test, in the tuberculoid few bacilli and positive Mitsuda test.

The widely differing characteristics of the three diseases just considered indicates that I do not suggest that they are precise analogues of sarcoidosis, but only examples of diseases in which immunological changes, in some sense peculiar to the individual patient, determine characteristic reaction to an infective agent. In this very general sense they illustrate w ys in which *M. tuberculosis* and other infecti agents could be concerned in the causation of sarcoidosis

T my mind, the evidence that in some cases sarcoidosis is related to *M. tuberculosis* infection is ery strong and the tw most important fields of study are the investigation of the nature and mechanisms of the immunological abnormality of the sarcoidosis-prone individual and the search for any other agents which may be ble to cause sarcoidosis in such individuals.

More intens immunological study of the Kveim test may be rewarding. Experience with this test is frequently regarded as evidence in favour of the hypothesis of causation by a specific unidentified agent and against any relationship to tuberculosis. But all the facts known about it—for instance, the clear difference in reactivity which it demonstrates between patients with caseating tuberculosis and with sarcoidosis—can be explained just as neatly by supposing that it detects the peculiar reactivity of *the individual* who is liable to develop sarcoidosis. Just as the Mitsuda test detects those individuals who in response to infection with *M. leprae* develop tuberculoid rather than lepromatous leprosy so the Kveim test might distinguish between those individuals whose response to mycobacterial infection is non-caseating epithelioid cell granulomatosis or sarcoidosis, and those whose response is caseating tuberculosis.

At present, of course, any hypothesis about the aetiology of sarcoidosis must be regarded as little more than an indication of opinion about the lines of research likely to be most rewarding. I advancing once again the arguments in favour of variant of one of the oldest hypotheses, I wish to make only tw points first, that it is illegitimate to exclude this or any other tenable hypothesis by definition, and secondly that in my opinion our knowledge of the causation of sarcoidosis is much more likely to be advanced by workers who keep their minds open, and examine all new evidence in the light of every tenable hypothesis than by those who adopt definition which excludes *priori* an important group of aetiological hypotheses.

References

Scadding, J G (1960) M. tuberculosis in the aetiology of sarcoidosis. Brit. med. J 2: 1617

Scadding, J G. (1962) The relations between sarcoidosis and tuberculosis. Bull. int. Un. Tub. 32 574.

Clinical Aspects of the Relationship Between Sarcoidosis and Tuberculosis

From the Institute of Diseases of th Chest Brompton, London, Great Britain

The Relationship of Sarcoidosis to Tuberculosis

J G Scadding

The relationship of infection with *M tuberculosis* to sarcoidosis cannot be discussed with anyone who regards the idea of any such relationship as so unthinkable that he excludes it by definition. The question of the role of *M tuberculosis* (or of any other agent) in the causation of sarcoidosis is to be decided by empirical study and not by the insertion of an arbitrary ordinance in a definition. I will assume therefore that we will agree later this afternoon to say nothing whatever about aetiology in our agreed definition of sarcoidosis.

The evidence indicating that there is a relationship of some sort between sarcoidosis and infection with *M tuberculosis* must be well known to you I summarised it in my Mitchell Lecture to the Royal College of Physicians of London in 1960 and again at a discussion at the 16th International Tuberculosis Conference in Toronto in 1961 so I will not weary you by going over old ground. I propose instead to consider briefly some hypotheses about the aetiology of sarcoidosis, and the role assigned by them to the immunological peculiarities which are such an important feature of this disease and then to discuss how in the light of these considerations, mycobacterial infection might be concerned in the pathogenesis of sarcoidosis.

The principal types of aetiological hypothesis and the roles assigned by them to immunological changes can be stated briefly as follows

Hypothesis I Sarcoidosis is due to an unidentified specific agent and the interaction between this agent and the host is the cause of the immunological peculiarities.

Hypothesis II Sarcoidosis is related to the collagenoses or to the reticuloses, in which immunological changes, in some respects resembling those observed in sarcoidosis, are prominent.

Hypothesis III Sarcoidosis occurs only in certain individuals who have a pre-existing immunological peculiarity and in them develops as a reaction to an agent, or one of several possible agents, which may or may not be known already as causing some well known disease.

The first two hypotheses clearly exclude any possibility of a direct aetiological role for *M tuberculosis* or any other known agent. The third admits several possible roles for an external causative agent. The range of possible roles can be illustrated by reference to some diseases in which relationships between host and agent of the sort which it postulates are well recognised.

Let us consider erythema nodosum, rheumatic fever and the two sorts of leprosy

Erythema nodosum is a syndrome with well-defined symptomatology clinical signs and course and with a uniform histological pattern in the eruption. But we know that it may be precipitated by infection with a variety of different agents *M tuberculosis, Str haemolyticus Coccidioides immitis, Histoplasma capsulatum* and others. Moreover it is not everyone who is infected with these agents who develops this reaction, but only certain individuals and at the appropriate stage of their reaction to the infection. And unless the right investigations are made at the right time the aetiological agent is unlikely to be demonstrated in an individual case, although

with regard to the frequency of supervening tuberculosis in sarcoidosis. There are two circumstances which can probably explain this difference. Previously when Boeck sarcoid was still considered to be a manifestation of tuberculosis, the treatment of these patients in sanatoria together with patients with infectious tuberculosis was not regarded as contraindicated. According to our present conception, however this meant that the patients were exposed to tuberculous infection, which may fully account for the numerous observations that the disease changed into tuberculosis. As a result of our present knowledge, nowadays we are anxious not to expose sarcoidosis patients to such risk of infection this may be one of the reasons why we now so rarely find the development of supervening tuberculosis in these cases. The fact that the risk of tuberculous infection in the general public is far less nowadays than it was, e.g. twenty years ago has a similar bearing.

Another important consideration is the selection of the material. Up to the 1940s our knowledge of lymphogranulomatosis benigna was based mainly on chronic and generalized cases. The risk of supervening tuberculosis was decidedly greater in cases of this type than in the benign and uncomplicated cases of early pulmonary sarcoidosis (cf. silicosis below).

The discrepancy in tuberculous complications, in material of the present day which are reported by various investigators from different sources, for example, from the Brompton Hospital and from St. Göran Hospital, can probably be accounted for by different method of selecting the material. At St. Göran Hospital this consisted mainly of early mild sarcoidosis (1 2,3 whereas it is likely that the Brompton material consisted, to a greater extent, of chronic and complicated cases.

Contrary to the previous opinion, that, owing to so-called positive anergy sarcoidosis patients have especially high resistance to tuberculosis, sarcoidosis is probably followed by a decreased resistance to tuberculosis. This assumption is supported not only by clinical observations but also by studies with radioactive BCG vaccine. When using this method, accelerated absorption was demonstrated in cases of advanced sarcoidosis compared with the absorption in mild cases (4) *This fact might be interpreted as the* result of a lowered immunity to tubercle bacteria in cases of advanced sarcoidosis.

In this respect it seems appropriate to make a comparison with silicosis. As is well known, tuberculosis is inclined to develop, to a large extent, in patients with silicosis. This fact has not been regarded as a reason for concluding that silicosis has a tuberculous origin. Moreover it is of interest to note that the risk of supervening tuberculosis *is decidedly higher* in patients with advanced silicosis than in those suffering from a mild form of the disease. Sarcoidosis can probably be considered from a similar point of view since, gross anatomically and histopathologically this disease is closely related to silicosis. It is only after the occurrence of an extensive blocking of the lymphatic system that there is a marked decrease in the resistance to concurrent tuberculous infection.

However tuberculous complications in cases of sarcoidosis do not consist only of the development of clinically manifest tuberculosis. In addition to the four above-mentioned cases of this type, in 12 out of the 700 cases (1.7%) in the St. Göran material, tubercle bacteria were found to exist in the excised lymph nodes or the gastric lavage fluid, without clinical or histopathological signs of tuberculosis. Such lack of agreement is very irritating when judging clinical and etiological data. It is, of course, possible that the tubercle bacteria in these cases were derived from an old, comparatively inactive tuberculous focus. The possibility cannot be excluded, however that the findings were due to contamination or to the samples being mixed up. Experience in connection with a material consisting of bronchial carcinoma is instructive in this respect.

In agreement with reports in the literature we have seen bronchial carcinoma develop in several patients with pulmonary tuberculosis. But in a few other cases of bronchial carcinoma, tubercle bacteria were found without any signs of clinical tuberculosis. Nor did subsequent autopsy reveal any pathologico-anatomical traces of tuberculosis. Con-

From St. Goran's Hospital, Stockholm, Sweden

The Relationship of Sarcoidosis to Tuberculosis

SVEN LÖFGREN

When discussing the relationship of sarcoidosis to tuberculosis, it would seem that we have to choose one of three lines, corresponding to the three concepts of sarcoidosis described in another paper (6)

According to the first theory sarcoidosis is an anergic or hypoergic form of tuberculosis, with high resistance to the tubercle bacterium, what Jadassohn terms "a positive anergy" This theory maintains that there are not two diseases, but only one, tuberculosis, which can appear sometimes in one form, sometimes in another The problem is then essentially which is the immune-biologic mechanism that may be responsible for this change, and why does the change occur

If we adopt the second concept of sarcoidosis, basing its definition on histopathologic criteria (8) we have three conditions to consider classical tuberculosis, tuberculous sarcoidosis, and sarcoidosis of unknown origin. This theory has still to explain why some cases of tuberculosis react with a sarcoid tissue reaction.

The third concept of sarcoidosis, which defines it on a clinical basis, postulates two in their origin independent diseases tuberculosis and sarcoidosis. They have an important relationship, however and this is easy to explain As both diseases are fairly common they sometimes occur in the same individual, either at different times or simultaneously (5) Thus, many sarcoidosis patients have a history of previous tuberculosis or at least, primary tuberculous infection, indicated by a positive tuberculin reaction or calcareous foci.

In 1952 a material consisting of 212 cases of sarcoidosis, mainly of an early type, was reported on from St. Göran's Hospital (2) In 80 of the cases (38 %) there were criteria of previous tuberculous infection some patients were spontaneously tuberculin positive at least one year before the occurrence of sarcoidosis in some cases, moreover the X-ray films revealed calcified foci other cases had a history of active tuberculosis, either exudative pleurisy or caseating tuberculosis.

In the literature however it is much more frequently mentioned that pulmonary tuberculosis may supervene on pulmonary sarcoidosis. According to Schaumann's experience in 1937 most patients with lymphogranulomatosis benigna died of classical tuberculosis (10) In a paper published in 1960 Scadding (9) quoted some studies made in recent years, which showed a decidedly lower frequency of supervening tuberculosis in cases of sarcoidosis. Thus, Riley (7) in 1950 reported the development of overt tuberculosis in 13 out of 52 cases of sarcoidosis in negroes in New York, and Uitvedt (11) in 1948, reviewing 59 reported necropsies in cases of sarcoidosis, found that tuberculosis was the cause of death in 11 of these patients. (For other references with a still lower frequency of supervening tuberculosis in cases of sarcoidosis, see Scadding.) In his material from the Brompton Hospital, Scadding observed five out of 230 cases (2) in which transition from sarcoidosis to caseating tuberculosis had taken place The corresponding frequency in a sarcoidosis material from St Goran's Hospital was four out of 700 cases (0.6)

Thus, there is considerable divergence between the older and the more recent literature

with regard to the frequency of supervening tuberculosis in sarcoidosis. There are two circumstances which can probably explain this difference. Previously, when Boeck's sarcoid was still considered to be a manifestation of tuberculosis, the treatment of these patients in sanatoria together with patients with infectious tuberculosis was not regarded as contraindicated. According to our present conception, however, this meant that the patients were exposed to tuberculous infection, which may fully account for the numerous observations that the disease changed into tuberculosis. As a result of our present knowledge, nowadays we are anxious not to expose sarcoidosis patients to such risk of infection; this may be one of the reasons why we now so rarely find the development of supervening tuberculosis in these cases. The fact that the risk of tuberculous infection in the general public is far less nowadays than it was, e.g. twenty years ago has a similar bearing.

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The discrepancy in tuberculous complications, in material of the present day, which are reported by various investigators from different sources, for example, from the Brompton Hospital and from St. Göran's Hospital, can probably be accounted for by a different method of selecting the material. At St. Göran's Hospital this consisted mainly of early, mild sarcoidosis (1, 2, 3), whereas it is likely that the Brompton material consisted, to a greater extent, of chronic and complicated cases.

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In view of the clinical and bacteriological data available some fifty years ago, it seems quite reasonable that Boeck's sarcoid was then interpreted as a manifestation of tuberculosis. The knowledge and experience of the disease, which we have gained during the last 10—15 years, makes it far more likely that sarcoidosis is a condition without etiological connection with tuberculosis. However in its relation to tuberculosis it can give rise to the same complications as those observed in connection with a number of other diseases of a non-tuberculous origin.

References

1 Lofgren, S. Acta med. Scand. *1 4* Suppl 174 1946.

2. Lofgren, S. and Lundbeck, H.: Ibid. *14* 259 265, 1952.

3 Lofgren, S. Ibid. *145* 424 465, 1953.

4 Lofgren S., Strom, L., and Widström, G.: Acta paed. *43* Suppl. *100* 160 1954

5. Lofgren, S. Trans. 4th Commonw Health and Tuberc. Conf. 1955: 78.

6. Löfgren, S. Proc. 3rd I tern. Conf. on Sarcoidosis. Acta med. Scand. Suppl. *425*. 3, 1964

7 Riley E. A. Amer Rev T berc. *62* 231 1950

8. Scadding J G Brit. med. J., 1950 745.

9. Scadding J G. Ibid. 1960 1617

10 Schaumann, J Nord. Med. Tidskr *13*. 745, 1937

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From Tok) H alth Institute, J pan National Railw ys, Japan

The Relationship of Sarcoidosis to Tuberculosis

Y Hosoda and Y Chiba

The relationship of sarcoidosis to t berculosis is to be discussed from the following fi points.

1) Time relationship of hilar adenopathy to tuberculin positi conversion.

3,838 fresh-tuberculin-converters, who had been detected among tuberculin-negative re actors by tuberculin testing t three months intervals, were X-rayed at the same intervals. 134 cases out of them revealed hilar node tuberculosis at the time of positiv conversion, only 2 cases during the first three months and none during period of three years after that. On the other hand, 14 cases w th sarcoidosis were already tuberculin-positive long before the onset, and 10 of these cases once lost the sensitivity during the course of the disease. Thus, it can be said that the time relationship of hilar adenopathy to tuberculin positiv conversion was quite differen between the t diseases.

2) Tuberculin sensitiv ty

The positive tuberculin rates according to ages were also much lower in the cases with sarcoidosis than those for the whole population.

3) Recent trend of new manifestation of hilar node tuberculosis and sarcoidosis.

Hilar node tuberculosis has decreased not only in the actual number of the cases but also in the rates of the cases with hilar node tuberculosis per the cases of primary tuberculosis. During the period of 1952 through 1959 there were 11 cases with hilar adenopathy discovered among about 40,000 male adult workers in Tokyo. 10 cases out of them, were sarcoidosis and only one was tuberculosis.

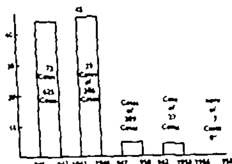


Fig. 1 Rates of Cases ith Hilar Adenopathy out of Primary Tuberculous Cases (Chiba & Hosoda et al.)

4) Age distribution.

The cases with bilateral hilar adenopathy due t sarcoidosis were found in an older age-group than those with bilateral hilar node tuberculosis.

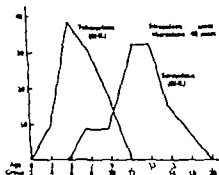


Fig. 2. Age Distribution of Cases with BHL due to Sarcoidosis and Tuberculosis Prinz-Regent Leopold Kinderheilstätte in West Germany (Chefarzt Dr med. J Heiland) during the period of 1940 through 1959. (Hosoda et al.).

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From Tokyo Health Institute, Japan National Railways, Japan

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Y. HOSODA and Y. CHIBA

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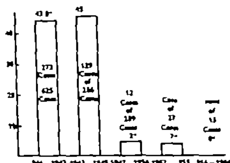


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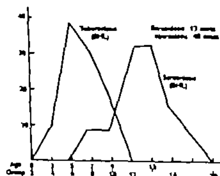


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3. Lofgren, S. Ibid. *145* 424 165, 1953
4 Lofgren, S., Strom, L., and Widstrom, G. Acta paed. *43* Suppl. *100* 160 1954
5 Lofgren, S. Trans. 4th Commonw Health and Tuberc. Conf. 1955 78.
6. Lofgren, S. Proc. 3rd Intern. Conf. on Sarcoidosis. Acta med. Scand. Suppl. *425* 3, 1964
7 Riley E. A. Amer Rev Tuberc. *62* 231 1950.
8. Scadding J G : Brit. med. J 1950 745.
9 Scadding, J G. Ibid. 1960 1617
10. Schaumann, J Nord. Med. Tidskr *13* 745, 1937
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This does not correspond to the Kveim test, which [illegible] in the ordinary reactive state seen in the positive Mantoux or other "late" skin reactions. Further, the fact that patients with tuberculosis fail to react to Kveim antigen whilst patients with sarcoidosis fail to react to whole mycobacteria unless Mantoux positive suggests that the antigenic material in these tests can be in no way closely related.

If Dr. Scadding's postulate that the Kveim test may reveal only [illegible] certain state of host reactivity in [illegible] non-specific sense were correct, it is difficult to understand why so many workers (and this is our own experience) have failed to elicit any true [illegible] reaction except in cases of sarcoidosis when using material from assured patients.

Dr. James. I hold the same view as Dr. Sven Löfgren and this I would now regard to be the more widely held opinion by most of us both at this conference and in Britain. One important factor in the emotionally charged argument of whether sarcoidosis is or is not related to tuberculosis is the type of material under investigation. The modern ophthalmologist and the modern dermatologist do not argue this problem, ... it is the chest physician who is the most likely person to confuse the two diseases and therefore tends to keep them together. This is not surprising since the chest radiograph is so similar in the later stages of sarcoidosis and tuberculosis.

Dr. Siltzbach. The slide which Dr. James has alluded to, Schema for Course of Sarcoidosis, was one that I presented last week in London (Fig. 1). This schema attempts to picture in time-table fashion the events which may possibly precede the clinical onset of the disease as well as three of its more common outcomes. On the bottom three lines I have sought to portray the immunologic state preceding the clinical illness, during it and after recovery, employing tuberculin and Kveim test studies. Also that, the three courses of varying duration and severity are shown: first, the mildest course lasting two years or so shown by solid line, with mediastinal lymph node enlarge-

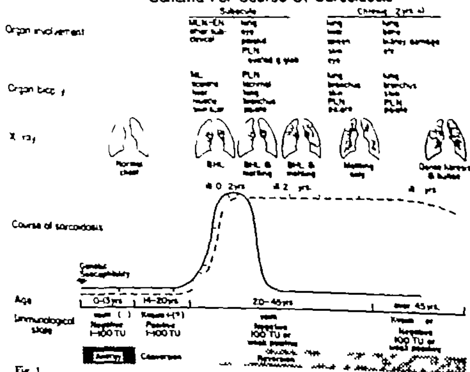


Fig. 1

5) X-ray findings.

In hilar node tuberculosis, 40 out of 1 198 cases had bilateral hilar lymphadenopathy while in sarcoidosis 44 of 45 cases had it.

From the above-mentioned results, the authors could not find any clue supporting the tuberculous origin of sarcoidosis.

References

1. Chiba Y. Clinical Study of Tuberculous Primary Infection Development of Tuberculosis, Hoken-Dojin-Sha Publishing Com. Tokyo, 1950.
2. Hosoda, Y. Proceedings of the 16th Assembly of Japan Medical Congress, III, 566, 1963.
3. Hosoda, Y. Japanese J. Chest Dis. 7. 4. 400, 1963.
4. Hosoda, Y. Japanese J. Chest Dis. 7. 3, 332, 1963.

DISCUSSION

DR SOMMER: We are now going to discuss these three important papers. I think it will be a difficult task and it seems to me that we have now reached one of the summits of this conference.

In 1960 when the last conference on sarcoidosis was held in the U.S.A., there was no discussion on the etiology of sarcoidosis. This does not surprise me. But discussions are now taking place in Europe, where there are some well-known investigators of sarcoidosis who still believe that sarcoidosis is a special form of tuberculosis. However, as long as we do not know the cause of sarcoidosis we have to discuss seriously tuberculosis as a possible cause of sarcoidosis. But there is one important point in such a discussion. What we believe or do not believe about this subject is of very little interest. What we need is the definite proof or refutation of the tuberculous etiology of sarcoidosis. As long as we have not obtained this proof we ought not to forget to emphasize that tuberculous etiology is still only hypothesis, and not a fact, as Dr Scadding has pointed out. Complete confusion ensues if some investigators, who assert tuberculous etiology recommend streptomycin plus cortison for the treatment of sarcoidosis. Thus, the good results obtained will not help us to decide whether tuberculosis or sarcoidosis was treated.

May I put two questions to Dr Scadding.

1. It is a fact that tuberculosis starts with primary infection and resulting primary complex. Do you think that the Lofgren-Syndrome is special form of tuberculous primary infection?

2. Did you ever observe sarcoidosis in young subjects who previously showed complete anergy to tuberculin, with this test becoming positive just at the onset of the Lofgren-Syndrome? We have never observed such case.

I think that further studies should be made especially on young persons who show complete natural tuberculin anergy just before the onset of the Löfgren-Syndrome.

DR ISRAEL: What percentage of patients who had tuberculin tests recorded prior to the onset of sarcoidosis were positive?

DR HOSODA: Most of the sarcoidosis cases were tuberculin positive.

DR REID: I would first like to make an attempt to answer Dr Lofgren's question on the nature of the sarcoid reaction (Concepts of Sarcoidosis). Firstly the intensity of tissue response to an antigen appears to be the resultant of a reaction between two variables the degree of hypersensitivity in the host and the amount of available antigen. Judged by experimental skin tests, the same reaction may be obtained by adjusting dosage or altering sensitivity. Infection with different organisms of different antigenic potency may thus give similar tissue reponses if the host reaction happens to be appropriate. Alternatively a single infection may result in different responses when there are differences in host reaction as seen in the different patterns—caseation or sarcoid-like—of tuberculosis. Secondly the histological sarcoid pattern is the result of a hypersensitivity reaction to an antigen in certain physical state, the chief determinant being probably particulate size, which evokes characteristic cellular response.

The sarcoid pattern is therefore not specific for any particular disease but rather as I tried to indicate the other day represents recognizably distinct late type of hypersensitivity reaction. In the skin this response is seen as both accelerated tubercle reaction, as the Mitsuda reaction and, I suggest, in the positive Kveim test. All of these reactions have comparable macroscopic appearances and time sequences and can be histologically identical.

If this is true, then the fact that the sarcoidosis patient will react to Kveim antigen in fashion comparable to the reaction of the tuberculous patient when given heat-killed, acid-fast bacilli, indicates that there is no depression of tissue reactivity in sarcoidosis provided that the correct antigen is used. The truly non-reactive state is seen in lepromatous leprosy and in non-reactive tuberculosis where cells are swarming with organisms and the cellular reaction is minimal.

CARDIO PULMONARY FUNCTION PROGNOSIS THERAPHY

Moderator: JOHN S. CHAPMAN

The Cardio-Pulmonary Function in Sarcoidosis

From the Department of Pulmonary Diseases, Medical Clinic, University Hospital, Groningen, Holland.

Obstructive Lung Disease in Pulmonary Sarcoidosis

G. J. TAMMELING, K. DE VRIES, H. J. SLUITER, N. G. M. ORIE, H. TEN HAVE, J. WITKOP and A. ZUIDERWEG.

Summary

In most of the longstanding — diffuse — cases of sarcoidosis with pattern of obstructive lung disease, the percentage of cases complicated by *preexisting independent chronic* aspecific respiratory affection (= group asthma — bronchitis) is very high[1]

In contradistinction to pure fibrotic cases *function* pattern is found of (partly) reversible obstructive lung disease.

This obstructive pattern is reflected in increase of residual volume (R.V. and F.R.C.) in increase of viscous work of breathing and in disturbance of *pulmonary mixing*. *It is* probably based on an increased reaction to aspecific stimuli (hyperreactivity) and sometimes on allergy.

This *complication* explains the unfavourable course of this type of disease and is probably the main cause for *the persistence of the* lesions in number of cases in which peripheral pulmonary spread occurs. It also explains that this development is much more common in males than in females, contrary to the general trend of sarcoidosis.

It explains the frequent bacterial infections in this type of disease, and the obstructive character of the C.A.R.A. explains the development of the emphysematous forms of sarcoidosis.

Consequently also cor pulmonale and diffuse bilateral bronchiectasis are practically pathognomonic for this double affection.

C.A.R.A. is Dutch for chronic aspecific respiratory affections and is the equivalent for C.N.S.L.D. (Fletcher). It includes asthma, asthmatic bronchitis, eosinophilic bronchitis, diffuse bronchiectasis and emphysema, and it is considered by us as an independent and usually preexisting entity mainly characterized by reversible bronchial obstruction and its sequelae on constitutional hereditary base.

For references, see ten Have, H. Klinische aspecten van de ziekte van Besnier-Boeck Schaumann. Thesis Groningen 1958.

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ment sometimes accompanied by erythema nodosum (MLN EN) sometimes with transient pulmonary mottling second, the dotted curve representing the course of patients with pulmonary mottling and extrathoracic sarcoidosis lasting two to seven years with slow healing, often with residual scarring and third, the most chronic course with the poorest prognosis—the course marked by dashes—lasting more than seven years with substantial organ impairment by scarring and continuous seeding with granulomas. Above these are listed organ biopsy and chest X ray findings under the two main headings of subacute and chronic phases of sarcoidosis.

What interests me most perhaps is the left or preclinical side of this schema. I use this schema to put forward the tentative proposal that sarcoidosis is a hypersensitivity disease affecting a genetically susceptible segment of our population which is larger than we realize, and that these individuals may somehow be prepared in childhood or youth by an infection with one of many varieties of mycobacteria or other unknown agents which act as an "adjuvant" in the sense of Freund adjuvant. When challenged at the age of 20—35 years by some internal or external agent, these hypersensitive individuals manifest a granulomatous explosion in their tissues which we call sarcoidosis. Dr Hosoda studies among Japanese railway workers in whom a positive tuberculin test preceded the onset of sarcoidosis is another study of which there have been several others, reporting this. What we need to nail this down are studies of sarcoidosis among populations in which positive tuberculin reactions are quite uncommon.

Dr Israel We went over Dr Hosoda s data. He has records of 14 patients with records of tuberculin tests prior to onset of sarcoidosis. Thirteen were tuberculin-positive, and 10 of these reactors were found to be tuberculin-negative after sarcoidosis had developed.

Dr Scadding Although one should not take sides and get emotionally involved in scientific studies, I was not unmoved when I heard Dr Siltzbach coming round to the view rather cautiously perhaps, that there may be some relationship between sarcoidosis and tuberculosis. My own opinions on this have changed over the years. I first became interested in sarcoidosis in 1937 as a result of two wonderfully clear lectures which Professor Snapper gave in London. These left me with the strong conviction that sarcoidosis had nothing to do with tuberculosis. It was the cumulative effect of clinical experience after the war which led me to my present viewpoint.

Dr Sommer asked about evidence of primary tuberculous infection in patients with Lofgren s syndrome. Certainly I have seen many patients with this syndrome who have old calcified residues. More striking, perhaps, are the patients who have been treated for pulmonary tuberculosis, and then develop a typical sarcoid bilateral hilar adenopathy sometimes within a short time after the active stage of their tuberculosis.

Dr Reid asked about the paradox, on the view that sarcoidosis can be related to tuberculous infection, that erythema nodosum can be associated both with the primary infection when skin reactivity is high and with the early stage of sarcoidosis when it may be low or absent. Here one can only speculate, of course. Presumably erythema nodosum is the result of an antigen-antibody reaction of some sort. It seems to me quite possible that the antibodies concerned with the production of erythema nodosum and of tuberculin skin reactions are of different types, and that while persons liable to develop sarcoidosis have for at present unknown reasons, deficient ability to produce one of these types of antibody they retain the power to produce the other

Table I. Sarcoidosis material

Group	Number of patients	Mean age	Reason for admittance		E.N.	Effort dyspnea	Orthostatic complaints	Duration of symptoms
			X-ray	Symptoms				
I	11	33,3 (21—46)	5	6	4	5	2	2—28
II	11	39,2 (29—58)	8	3	0	5	5	2—50
III	15	44,3 (29—54)	3	12	0	14	1	48—279

It is not astonishing that patients with lung fibrosis are breathless, but why dyspnea in patients with BHL? W have heard about pronounced orthostatic changes in serum proteins in these patients. Perhaps in pulmonary sarcoidosis orthostatic factors with increased effect of gravitational shifts of the blood could explain their breathlessness or are these entilatory or diffusional disturbances also in those patients, where w cannot see any pulmonary changes on X-ray

Physiological Studies

The aim of these investigations (Holmgren and Svanborg 1961 Svanborg 1961 Svanborg 1961 Holmgren and Svanborg 1964) has been to describe the effect of the sarcoidotic lesion on the cardio-pulmonary function. As an overall test of this function the oxygen transport capacity expressed by the rate of work that can be performed at heart rate of 170 beats/min, W_{170}, can be used (Sjöstrand 1947 Holmgren 1962) This measure expresses the work pulse which is approximately the same as the oxygen pulse and thus equal to the product of the AV oxygen differences times the stroke olume. W_{170} is related to sex, body size and physical fitness and can be determined for instance during exercise on bicycle ergometer This submaximal measure has been found to be highly correlated to such anthropometric parameters as, heart olume, and total hemoglobin (Kjellberg, Rudhe, Sjostrand 1949)

W_{170} was determined in the present studies on bicycle ergometer and related to both THb and heart olume (Fig. 1 and 2) As is seen from these figures, pulmonary sarcoidosis, irrespective of stage of disease is accompanied by low exercise tolerance, the verage lying between minus one and two times the standard deviation for the normal relationship between these tw parameters.

T answer the question which component in the oxygen conduction line, entilation, diffusion or circulation, explains this limitation of the oxygen transport capacity of the sarcoidotic patient, thorough analysis of the cardio-pulmonary function at rest and during excercise was performed. Pulmonary function was studied with static and dynamic spirometry determination of dynamic compliance, total lung resistance, alveolar and arterial gas tensions and steady state diffusion capacity during exercise in upright position The circulation was studied after heart catheterization, with determination of pressures and flow in the pulmonary and systemic circulation, at rest and during exercise in supine.

Static and dynamic spirometry fig. 3, 4 and 5 (Svanborg 1961) showed that static lung volumes were decreased in all stages of the disease, and more so in the severely fibrotic patients. This group also had an increased residual quotient. *Dynamic spirometry* showed decreased alues and *total lung resistance* increased alues in all groups. The *gas exchange* at rest and during moderate exercise, as expressed by

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Disability in Sarcoidosis[1]

A. Holmgren and N. Svanborg

This report discusses shortly the type and the degree of the functional disturbances in pulmonary sarcoidosis, with special reference to the different stages of the disease.

In our clinical work we use physiological tests for different purposes, among others for an early detection of specific functional picture of the disease, to judge if, from a functional point of view there are more or less pronounced indications for therapy and to get an objective measure of the result of treatment.

For a clear understanding of the symptomatology and course of pulmonary sarcoidosis it is necessary to study the different types of the disease both clinically and from a functional point of view.

Material

The division of the material into groups was made according to the clinical course of intrathoracic sarcoidosis. Only cases where the clinical diagnosis was supported by lymph node biopsy have been studied. Guinea pig tests from lgl and gastric lavage were negative.

Some significant data have been collected in the table.

Group I Patients with bilateral hilar lymph-node (BHL) enlargement but without lung lesions in X ray

Group II Patients with more or less pronounced lung parenchymal lesions but without signs of fibrosis.

Group III Patients in a chronic stage with different degrees of pulmonary fibrosis, a. slight, b. pronounced fibrosis.

The mean age increases in the groups with increasing lung lesions. The ranges show that there are great variations within the groups and also in the fibrosis group some patients are less than 30 years old, though they already have got severe lung fibrosis. In group III the males as a rule are younger than the females. The observed sex-difference might be accidental but a possible explanation could be a more rapid course in the males. Clinically one has the impression that though the females dominate in the earlier stages, the males have a somewhat higher frequency of lung fibrosis.

In Sweden many of the sarcoidosis patients are asymptomatic and are detected in routine X-ray but we can see in these small groups that already in the earlier stages the reason for admittance often is symptoms of the disease.

In the first group with BHL, except the Erythema Nodosum cases, effort dyspnea is a common symptom. Some of the individuals in this group and several in the "parenchymal" group II complain of orthostatic symptoms. In group III effort dyspnea is the main symptom.

[1] This work has been supported by grants from the Swedish National Associations against Heart and Chest Diseases.

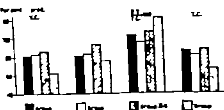


Fig. 3. Lung volumes in per cent of predicted values (Svanborg, 1961) mean values for the three groups.

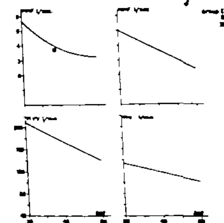


Fig. 4. Dynamic spirometry in sarcoidosis. Volumes in l BTPS. MMF l/sec. = maximum midexpiratory flow MVV l/min. = maximum voluntary ventilation at free respiratory rate. Full line indicates normal regression reported by Grimby et al (1961). Broken lines indicate lower normal border in upper figures and S. D. in the lower ones. (Svanborg 1961)

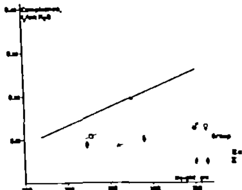


Fig. 5. Compliance, l BTPS/cm H_2O in relation to body height, cm. Straight line indicates normal regression obtained with the same technique by Ehrner 1960. Broken lines indicate S.D. Mean values are given for the different groups. (Svanborg, 1961)

arterial oxygen saturation and the partial pressures for oxygen and carbon dioxide, was normal in the BHL cases; in group II one subject had decreased arterial oxygen tension and saturation, caused by a large right to left shunt measured during oxygen breathing (16 per cent of the cardiac output). In the fibrotic groups arterial oxygen tension and saturation were reduced in two subjects at rest and in five subjects during moderate exercise.

The hydrogen ion concentration in arterial blood was normal at rest in all subjects, as was the arterial carbon dioxide tension and standard bicarbonate, indicating that alveolar ventilation at rest was capable of a normal CO excretion. During moderate exercise partly compensated metabolic acidosis of a normal magnitude, related to the work load, appeared in group one and two, and only in group three did marked metabolic acidosis develop during moderate exercise. No subject had an abnormal standard bicarbonate at rest, indicating that no subject hypo-or hyperventilated chronically. One subject with severe fibrosis hypoventilated during exercise.

The diffusing capacity of the lungs for carbon monoxide, $D_{L_{CO}}$, fig. 6, was markedly decreased in all subjects if corrected for body size and age. Also in group one without parenchymal lesions, this was the case, probably indicating pulmonary lesion although invisible on X-ray.

The influence of sarcoidosis on the ventilatory function, is mainly *restrictive*, decreasing the lung volumes, and *moderately obstructive* probably by increasing the *viscous resistance* of the lungs and by impairment of the conductance of the airways.

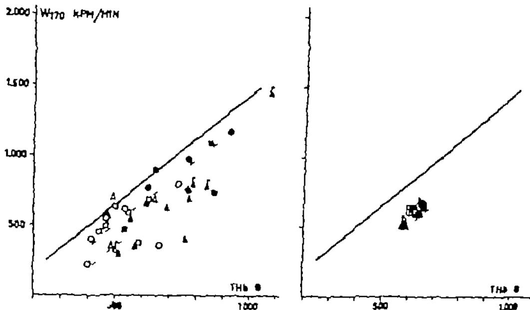


Fig 1 Rate of work at a heart rate of 170 cpm W_{170} in relation to total hemoglobin, THb, g. Full line indicates normal relationship between these two parameters, Holmgren et al. 1960, broken lines, SD Filled circles, men in group I open circles women, group I filled squares men group II open squares women group II Filled and open triangles indicate men and women in group III. Flag on triangle indicates slight fibrosis. The right figure gives the mean values for the groups.

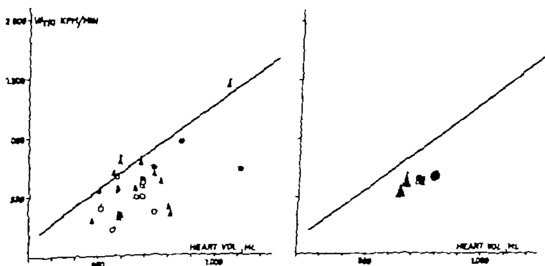


Fig 2 W   kpm/min, in relation to roentgenological heart volumes in   pine Symbols as in fig 1

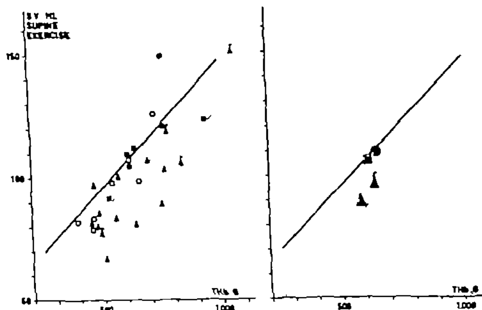


Fig. 8. Stroke volume, ml., during exercise in supine in relation to THb. Full line indicates normal regression found between these two parameters (Holmgren et al. 1961) broken lines, S.D. Symbols as in fig. 1

with larger Q. This implies also that the arteriovenous oxygen difference varies normally with oxygen uptake.

The *stroke volume* SV during exercise, was normal in relation to total hemoglobin (fig. 8) i. e. in relation to body size, sex and fitness, in group I and II but significantly low in group III and IV

The *pulmonary vascular* resistance was normal in group I and II but significantly increased in group III

Circulatory changes in supine thus occur late in patients with sarcoidosis and can shortly be described as decrease in stroke volume and increased pulmonary vascular resistance in the fibrotic stage.

Let us now turn back to the primary observation that patients with pulmonary sarcoidosis have decreased working capacity and try to explain this observation with the aid of the findings presented above.

The measure of working capacity used, W_{170} states that the rate of work these patients can perform at heart rate of 170 beats per min. is decreased. It is well known that at work loads increasing V_{O_2} to above 800 ml, STPD/min. the interindividual variation in V_{O_2} at given load, is small. Under these circumstances work load $\approx V_{O_2}$. According to Fick equation,

$$V_{O_2} = \dot{Q} \cdot AVD = SV \cdot F \cdot AVD$$

where F = heart rate, beats/min and AVD arteriovenous oxygen difference ml/l. Consequently

$$\frac{V_{O_2}}{170} \approx \frac{W}{170} = SV \cdot AVD = SV \cdot (C_{a_{O_2}} - C_{\bar{v}_{O_2}})$$

where $C_{a_{O_2}}$ and $C_{\bar{v}_{O_2}}$ are the arterial and mixed venous oxygen contents. In other words, W_{170} is determined by the stroke volume and the arteriovenous oxygen difference. A heart lesion would affect W_{170} by impairing the stroke volume lung lesion such as sarcoidosis primarily by affecting $C_{a_{O_2}}$ and possibly secondarily by affecting the stroke volume.

In the present material the oxygenation of arterial blood is impaired only in one subject

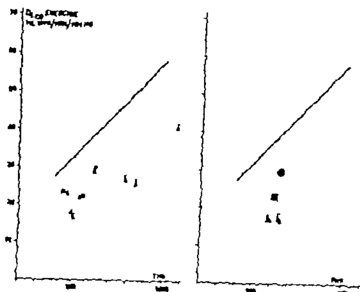


Fig. 6. D_{LCO} ml STPD/min/mm Hg during exercise in sitting position at a heart rate of approximately 140 cpm. in relation to THb, g Full line indicates normal regression in healthy young men and women (Holmgren, 1965) Symbols as in fig 1

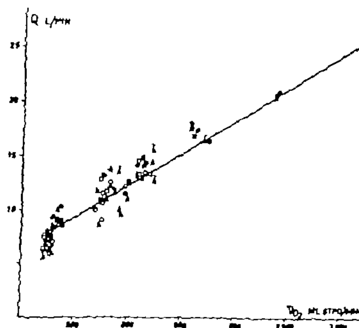


Fig 7 Cardiac output in supine, l/min. $\dot{Q}$ in relation to oxygen uptake, ml STPD/min., $\dot{V}_{O_2}$. Full line indicates normal regression in supine and open line S.D (Holmgren et al. 1961 Bevegård et al. 1961) Symbols as in fig 1

Alveolar gas exchange at rest is not severely impaired to the extent that the overall function of the lung as a gas exchanger at rest is decreased, until the patient has reached a stage with severe fibrosis. During exercise with increasing demands on the conductance of the lungs for oxygen such an impairment is common only in the fibrotic stage.

For an evaluation of the circulation, the subjects were studied at rest and during exercise in supine with determination of cardiac output ($\dot{Q}$) In fig 7 $\dot{Q}$ is related to oxygen uptake. The full line indicates the normal relationship found in healthy young men and women (1)

As is seen $\dot{Q}$ is normal in all groups at rest. During exercise cardiac output increases normally with increasing V_{O_2} If anything, the circulation tends to be slightly hyperkinetic, i. e. the same amount of oxygen is transported

Dr. Lofgren: Dr. Holmgren and Dr. Svanborg reported on the orthostatic reaction as a characteristic phenomenon in the cardio-pulmonary tests of patients with sarcoidosis. I heard her report the other day. Dr. Renée Norberg mentioned similar orthostatic influence on the serum proteins of sarcoidosis patients. I would like to know if there is a common explanation of these findings. In other words, is sarcoidosis principally linked with a tendency to orthostatic reactions?

Dr. Svanborg: In the discussion about the cause of the observed orthostatic reactions in patients with sarcoidosis and the observed serum protein decrease in the same patients (S. Lofgren), I should like to make some remarks. The orthostatic reaction could be studied as a pulse reaction in standing position at rest, and at work in different positions, and also be measured as the orthostatic influence upon the serum protein level. There is no specificity in the orthostatism in sarcoidosis. Every human being, though quite normal, has a slight tendency to orthostatic displacement of the central blood volume in upright position. The reason why this reaction is pronounced in sarcoidosis is not clear, but the orthostatic reaction can explain several symptoms in early sarcoidosis as tiredness and dyspnoea. Thus also in pulmonary disease the explanation of dyspnoea can be circulatory. If the decrease in central blood volume could be explained by changes in the diseased lung, by neuroregulatory disturbances, by altered intrathoracic pressure influence or other reasons is not known yet.

Dr. Scadding: I would like to put a question to Professor Orie. In coal-miners, a number of studies have shown that respiratory disability correlates well with a history of symptoms of cough, sputum and recurrent infections of the chronic bronchitis type, but hardly at all with radiographic category of pneumoconiosis. Are not the results Professor Orie has presented a similar phenomenon in sarcoidosis?

Dr. Svanborg: In the Dutch material presented by professor Orie many patients with pulmonary sarcoidosis showed a functional impairment of the obstructive type and also clinically had a story of *chronic bronchitis, asthma or emphysema*. In our Swedish material, patients with sarcoidosis as a rule have no signs of airway obstruction. Even in cases with severe lung fibrosis, *increased total lung resistance and delayed N_2-wash-out* time, the lung volumes are small and it is striking that the values for FEV are generally at normal. One explanation of the differences between the materials could be that we have no Dutch patients in our material and that Swedish people in general, and also sarcoidosis patients, seldom have symptoms of chronic bronchitis. There is a difference between our populations. Patients who already have asthma, emphysema, or chronic bronchitis can of course also get sarcoidosis but then the functional findings are not primarily due to their sarcoidosis. A study of the functional impairment caused by sarcoidosis should of course be made in patients without complicating disease. In our material asthma, emphysema etc. certainly are complications, but in the form which Orie describes they are seldom seen here.

Dr. James: Patients with sarcoidosis do not appear to have an increased incidence of bronchial infections until it is 20 years too late to do anything about it. We must try to detect and manage the patient long before he is subject to repeated respiratory tract infections.

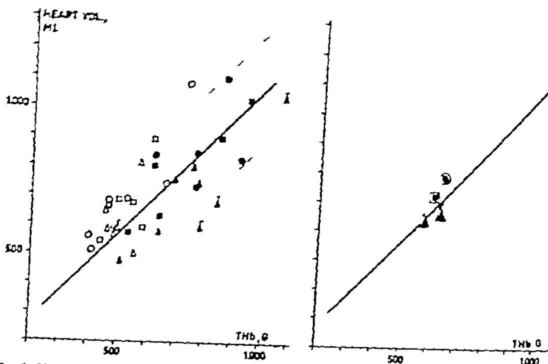


Fig. 9 Heart volume, ml, in supine in relation to THb, g Full line, normal regression between the parameter (Holmgren et al. 1961) broken lines, S.D Symbols as in fig 1

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References

1 Bevegård S., Holmgren, A. and Jonsson, B. Acta physiol. Scand. 49 279—298, 1960.

2 Ehrner, L. Acta med. Scand. Suppl. 353, 1960

3 Gadby G and Söderholm, B. Personal communications, 1961

4 Holmgren A., Jonsson, B. and Sjöstrand, T Acta physiol. Scand. 49 343—363 1960

5. Holmgren A. and Svanborg N Acta med. Scand. Suppl. 366, 7—38, 1961

6. Holmgren A. Scientific seminar arranged by Riksidrottsforbundets poliklinikkommitte, 1962

7 Holmgren A Svanborg, N I print. 1964

8 Kjellberg S R. R dhe, U and Sjöstrand, T Acta radiol 31 113—122 1949.

9 Sjöstrand T Acta med. Scand Suppl. 196. 687 -699 1947

10. S anborg, N Acta med Scand. Suppl 366. 39 74 1961

11 S nborg N A ta ed. Scand. Suppl. 366. 75 117 1961

12 Wurm K Reindell J nd Heilmeyer L. Der Lungen-Boeck m Rontgenbild, Georg Thieme Verlag 19 8.

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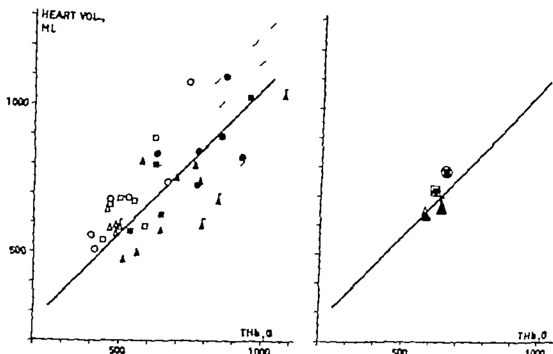


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References

1 Bevegård S. Holmgren A. and Jonsson, B. Acta physiol. Scand 49 279—298, 1960.
Eirnner, L. Acta med. Scand Suppl. 353, 1960
3 Grimby G and Soderholm, B. Personal communications, 1961
4 Holmgren A., Jonsson B. and Sjostrand, T Acta physiol. Scand. 49 343—363, 1960
5 Holmgren A and Svanborg N Acta med. Scand. S ppl. 366, 7—38, 1961
6. Holmgren A Scientific seminar arranged by Riksidrottsforbundets poliklinikkommitte, 1962
7 Holmgren A Svanborg N In print. 1964
8 Kjellberg S R Rudhe L and Sjostrand T Act radiol. 31 113—122 1949.
9 Sjostrand, T Acta med. Scand Suppl. 196. 687—699, 1947
10 Svanborg, N Acta med. Scand S ppl 366. 39—74 1961
11 Svanborg N Acta med. Scand Suppl 366, 75—117 1961
12 Wurm, K. Reindell, J and Heilmeyer L. Der Lungen-Boeck in Rontgenbild, Georg Thieme Verlag 1958.

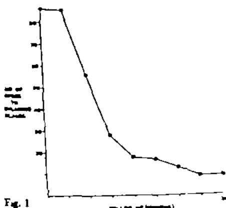


Fig. 1

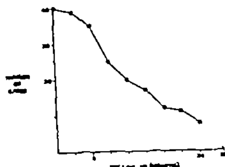


Fig. 2

Pulmonary Opacities

The 3 to 1 sex ratio was the same in the group of pulmonary opacities (F 48 M 16) In this group evidence of previous tuberculosis was found only half as frequently (6) as in the hilar gland group. The incidence of ey lesions was approximately the same in the tw groups, but erythema nodosum and superficial lymphadenopathy were much less frequently associated with pulmonary opacities than with enlarged hilar glands (17 against 39 for erythema nodosum 5 against 24 for superficial lymphadenopathy) There was, however more evidence of disseminate lesions (present in 14) All associated sarcoid manifestations were transient, with the exception of 1 case of kerato-conjunctivitis and 2 cases of bone involvement. Bone cysts became evident as unexpected findings in 2 patients who had clearing pulmonary opacities. Although the inference is that the disease is hronically active in these tw patients there ha e, in fact, been no further clinical manifestations of activity and in particular no relapse of pulmonary lesions.

Spontaneous clearing occurred in 40 cases (Fig. 2) Fifty per cent had cleared within 1 year and 80 % within 2 years. The remaining 8 (20 %) finally cleared in periods from 3 to 7 years. Fiv of the 8 patients had some slight residual pulmonary fibrosis not associated with respiratory disability Again there was no difference in the patients with and without erythema nodosum when the cases taking more than 2 years to clear were excluded, the respective times for clearing of pulmonary opacities being 10.9 months and 11.3 months. However none of the patients who demonstrated slow clearing had presented with erythema nodosum in this series.

Corticosteroid therapy was employed in ?3 patients with pulmonary opacities which were worsening over 6 months of observation or were unchanged at 1 year (Fig. 3) In one patient (T C) with associated kerato-conjunctivitis the ey lesions compelled the use of corticosteroids from the start.

There was no relapse after withdrawing treatment in 11 patients. Treatment was given from 6 to 25 months in this group. In 3 patients (N McB., N S., and E. J) there was relapse after withdrawing corticosteroids but treatment was not resumed. Tw went on to clear spontaneously In the 3rd (E. J) woman of 76, treatment proved impossible through lack of co-operation. She is a case of chronic sarcoidosis and has persisting pulmonary opacities, but no espiratory disability so far A single relapse occurred in 2 (H. W and E. N) but there was eventual clearing with further treatment, with no relapse on withdrawal. Five (P B., T C., L. M., D D D F) are cases of chronic sarcoidosis with continued activity of the disease over 3 1/2 to 7 years. These are all males, 2 of whom first presented after the age of 40.

The Prognosis of Early Sarcoidosis With and Without Erythema Nodosum

From the Dept. of Respiratory Diseases, University of Edinburgh, Great Britain

The Prognosis of Early Sarcoidosis

A. C. Douglas

I have taken "early sarcoidosis" to mean the hilar lymphadenopathy syndrome with and without erythema nodosum and pulmonary opacities not associated at the outset with manifestations suggesting long-standing disease. My remarks refer to 86 patients with enlarged hilar glands and 66 with pulmonary opacities who attend the Chest Clinics in Edinburgh and who have been observed sufficiently long to permit an assessment of prognosis. Follow-up in these cases has ranged from 1 to 11 years.

Enlarged Hilar Glands

The usual female predominance was noted (F 63 M 23). Thirteen per cent had evidence of previous tuberculosis varying from the radiographic appearances of healed primary lesions (the majority) to proven tuberculous pleural effusion and florid post primary disease.

Associated manifestations of sarcoidosis included erythema nodosum (39) superficial lymphadenopathy (mainly cervical) (24) and eye lesions which proved to be transient (5 %)

Fig 1 shows the number of cases with persisting hilar gland enlargement at various stages in follow-up. By th 12th month of observation spontaneous regression was complete in 68 of the 86 cases (79) and at 24 months 89 had resolved. Of the remaining 9 patients (11) 8 have shown persistence of enlarged glands without other manifestations developing over periods from 2 to 11 years. In only 3 of these patients with chronically persisting glands was the age of onset under 38 years. In the other 5 (F 4 M 1) the age at onset ranged from 38 to 59 substantially older than the average age for the group (30.5 years) The 9th patient developed pulmonary opacities and the disease is still active at 8 years.

In 12 of the 86 cases (14) pulmonary opacities appeared as transient phenomena as the glands began to regress. In all of these the opacities cleared spontaneously by the time the glands resolved completely over 3 to 20 months.

None of the patients with chronically persisting glands had presented with erythema nodosum. Excluding these cases, however there was no difference between the groups with and without erythema nodosum as far as time for resolution was concerned. In each group the average time to achieve complete regression was just under 8 months.

Glands which resolved spontaneously did so progressively and did not recur in subsequent follow up except in 1 case. This female patient showed initial reduction in size of glands at 7 months, with relapse at 12 months before final resolution at 23 months, with no drug treatment or intervening pregnancy to explain this behaviour

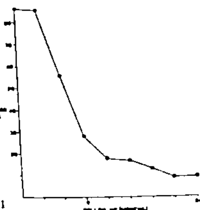


Fig. 1

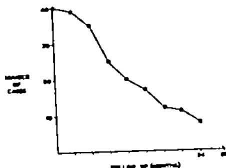


Fig. 2

Pulmonary Opacities

The 3 to 1 sex ratio was the same in the group of pulmonary opacities (F 48 M 16). I this group evidence of previous tuberculosis was found only half as frequently (6) as in the hilar gland group. The incidence of eye lesions was approximately the same in the two groups, but erythema nodosum and superficial lymphadenopathy were much less frequently associated with pulmonary opacities than with enlarged hilar glands (17 against 39 for erythema nodosum 5 against 24 for superficial lymphadenopathy) There was, however more evidence of disseminate lesions (present in 14) All associated sarcoid manifestations were transient, with the exception of 1 case of keratoconjunctivitis and 2 cases of bone involvement. Bone cysts became evident as unexpected findings in 2 patients who had clearing pulmonary opacities. Although the inference is that the disease is chronically active in these tw patients there have, in fact, been no further clinical manifestations of activity and in particular no relapse of pulmonary lesions.

Spontaneous clearing occurred in 40 cases (Fig. 2). Fifty per cent had cleared within 1 year and 80 % within 2 years. The remaining 8 (20 %) finally cleared in periods from 3 to 7 years. Fiv of the 8 patients had some slight residual pulmonary fibrosis not associated with respiratory disability Again there was no difference in the patients with and without erythema nodosum when the cases taking more than 2 years to clear were excluded, the respective times for clearing of pulmonary opacities being 10.9 months and 11.3 months. However none of the patients who demonstrated slow clearing had presented with erythema nodosum in this series.

Corticosteroid therapy was employed in 23 patients with pulmonary opacities which were worsening over 6 months of observation or were unchanged at 1 year (Fig 3) I one patient (T C.) with associated kerato-conjunctivitis the ey lesions compelled the use of corticosteroids from the start.

There was no relapse after withdrawing treatment in 11 patients Treatment was given from 6 to 25 months in this group. In 3 patients (N McB., N S., and E. J) there was relapse after withdrawing corticosteroids but treatment was not resumed Tw went on to clear spontaneously In the 3rd (E J woman of 76, treatment proved impossible through lack of co-operation She is case of chronic sarcoidosis and has persisting pulmonary opacities, but no respiratory disability so far A single relapse occurred in 2 (H. W and E. N) but there was eventual clearing with further treatment, with no relapse on withdrawal. Fiv (P B., T C., L. M., D. D D F) are cases of chronic sarcoidosis with continued activity of the disease over 3 1/2 to 7 years. These are all males, 2 of whom first presented after the age of 40.

Fig. 3

TABLE I Sarcoidosis Fate of Pulmonary Opacities 66 cases

Cleari g — Spontaneous or w th Corticosteroids	55 (83.5 *)
Relapse after Corticosteroids T eatment not resumed	1
Conti uing on Cort costeroids — Chroni Sarcoidosis	6 (9)
Cont uing on Corticosteroids (< 12 months treatment)	1
No treatment Considerable Pulmonary F brosis (Respiratory Disability n one)	3 (5)

* No Respiratory Disabil ty

A sixth case (F W) also a male is still active after 2 years of treatment and can probably also be included in this group. Two of these 7 chronic cases had presented th erythema nodosum. One patient (F McL.) has had less than 12 months of treatment.

In only 3 cases in the series was there im portant residual pulmonary fibrosis (T ble) None of these patients had been treated w th corticosteroid drugs only 1 has respiratory disability

In summary then 80 of hilar glands resol ed spontaneously within 12 months. A further 10 % resolved over the next year

Over 80 % of pulmonary opacities cleared either spontaneously or with corticosteroids (Table)

In sub-acute transient sarcoidosis the association of erythema nodosum did not appear to influence the rate of resolution of hilar glands or pulmonary opacities.

About 1 in 10 cases in both the hilar gland and pulmonary opacity groups have proved to have chronic sarcoidosis.

In general, in the hilar gland group, the older the age of onset the greater the likelihood of the condition persisting chronically

In the pulmonary opacity group males ha e fared orse than females as far as the tendency to chronicity is concerned but fortunately so far the use of long-term corticosteroid therapy has prevented pulmonary disability in these cases.

In a series of 66 patients w th pulmonary opacities only 1 untreated pat ent has important disturbance of pulmonary function. This could probably ha been a oided by suppressive corticosteroid therapy

From the Department of Tuberculosis of the University Medical School, Debrecen, Hungary

The Prognosis of Early Sarcoidosis With and Without Erythema Nodosum

László Mándi

Up to the middle of 1963 we observed 82 cases of thoracic sarcoidosis in Eastern Hungary. 14 of these cases were diagnosed as being due to associated erythema nodosum. It seems that thoracic sarcoidosis is often associated with erythema nodosum not only in Sweden and England but also in Hungary.

The majority of the patients with erythema nodosum were between 20 and 40 years of age. But thoracic sarcoidosis and erythema nodosum occur also in childhood. The diagnosis of sarcoidosis was confirmed by biopsy of the tonsils and prescalene lymph nodes in a ten-year-old girl, and by thoracic lymph-node biopsy obtained by mediastinoscopy in a girl of 13.

The significance of the hormonal factor was evident in the development of sarcoidosis and erythema nodosum. 12 patients were females. Erythema nodosum and thoracic sarcoidosis were observed in 3 patients after delivery and in one after lactation.

In 9 patients out of 14 the skin tests were negative to 1:100 old tuberculin, in 3 cases they were positive to 1:100 dilution and only in 2 patients was the test positive to 1:10,000 diluted tuberculin. Cultures for the demonstration of tubercle bacilli gave negative results in all cases.

The first clinical sign of the disease was the tumour-like enlargement of the thoracic lymph nodes in 11 patients, and, in addition to these symptoms, disseminated foci were observed in the lungs of 3 patients. Both these processes belonged to the early period of thoracic sarcoidosis. The contrast between the grave X-ray findings and the good general condition of the patients was impressive.

On the basis of the clinical picture, the X-ray and laboratory findings, and the course of the disease, these cases were diagnosed as sarcoidosis. In 8 cases the diagnosis was supported by the histological findings in connection with the following biopsy material: prescalene lymph node, tonsil, bronchial mucous membrane, thoracic lymph node obtained by mediastinoscopy or by thoracotomy, biopsy of the lung, Kveim test or excision from the muscle of the legs.

Seven patients recovered spontaneously. As regards the other cases, in 3 patients complete regression and in 2 satisfactory regression was obtained as a result of prednisolon treatment administered simultaneously with antituberculous medication. Two patients are still under treatment.

The great majority of our *entire material*, 78 cases out of 82, belonged to the early stage of the disease. When comparing the course of early sarcoidosis with or without erythema nodosum, no significant difference could be found between the two groups. The BHL syndrome showed a good prognosis, but when the lesions extended to the lungs cortisone therapy was also necessary in both groups. Perhaps the decisive factor was not the appearance of erythema nodosum or the increased allergic state, but the entire early stage of the disease and the fact that the patients were young.

We will now briefly describe some of our cases.

The first case was a girl of 13. Her parents and th other seven children wer healthy In 1961 erythema nodosum appeared and the tumour like enlargement of th thoracic lymph nodes was established by X ray examination The Mantoux test in 1 100 dilution was negative. Daniels biopsy and biopsy of the bronchial mucous membrane performed in a rural hospital were found to be negative. Despite this, the disease was considered to be sarcoidosis, and the patient was given a short cortisone treatment, and, subsequently treatment was continued for two months. On both occasions the X-ray findings showed considerable regression, but a relapse ensued after the drugs had been withdrawn for a period of two months. After this the girl was taken to our department.

The diagnosis of sarcoidosis was confirmed by the histological finding of thoracic lymph nodes, obtained by mediastinoscopy and the nodule produced by Siltzbach Kveim material. Prolonged cortisone therapy resulted in complete egression.

The second case was a girl of 10. At the end of 1958 after the ppearance of erythema nodosum, the patient had fever and a cough. Disseminated foci and the BHL syndrome were observed in the lungs at a rural hospital. Streptomycin and isoniazid treatment was carried out for half a year with no results. As the tuberculin test and the bacteriological examination for tuberculosis gave negative esults, the girl was sent to our depart ment for further examination. The diagnosis of sarcoidosis was confirmed by the histological findings in regard to the biopsy specimens from th tonsil and prescalene lymph nodes. A short prednisolone cure lasting 4 months resulted in considerable regression, but a relapse occurred after half a year Prednisolone treatment for one year gave rise to considerable regression, which seems to be permanent. The patient has now been under observation for 3 years.

The third case was a 29-year-old woman. She gave birth to a child in 1951 and seven months after lactation had ceased, erythema nodosum ppeared on the skin of her legs. A typical form of the BHL syndrome was revealed by X-ray examination. Sh had no fever The tuberculin test and cultures for tubercle bacilli were repeatedly negative. Biopsy was not performed then. Since we believed that the disease was sarcoidosis, she was kept under observation without treatment. She recovered spontaneously within a year The period of observation is now twelve years.

The fourth case was a woman aged 26. While in good health, erythema nodosum ppeared on the skin of her legs in 1958. X-ray examination showed the tumour-like enlargement of the thoracic lymph nodes and a slight dissemination in the lungs. The histological findings by Daniels biopsy showed the characteristic ppearance of sarcoidosis. It was suggested that for the time being she should be kept under observation. The physician at the dispensary gave her streptomycin and isoniazid treatment for 3 months, without obtaining any results. After this, erythema nodosum reappeared and a larger number of foci were observed in the lungs she was readmitted t our depart ment. Prednisolone treatment for one year resulted in considerable regression, which seems to be permanent. The case has now been followed up for 4 years.

It must be admitted that the number of our erythema nodosum cases is small 14 in all. Consequently we are only justified in speaking of our impressions" and make no claims to statistically confirmed conclusions.

From St. Göran's Hospital, Stockholm, Sweden.

The Prognosis of Early Sarcoidosis With and Without Erythema Nodosum

SVEN STAVENOW

In the literature on sarcoidosis it is customary to classify sarcoidosis with erythema nodosum (E. N.) as an acute complaint, and several investigators have shown that this early acute type of sarcoidosis has good prognosis. In some 75—90 % of the cases there is complete recovery within tw years after the onset of the disease (4 6—7 9 1 3)

However with regard to the prognosis of sarcoidosis without E. N contradictory statements ha been made (6, 2, 5 As has been pointed out this can be explained by several factors. Differences in classification and selection of patients, and racial and geographical variations are important factors in this connection. I number of the series published, acute, subacute, and chronic cases were taken together without the real onset and duration of the disease being known.

The *aim of this study was to ascertain* whether there is any difference, as regards prognosis, between sarcoidosis cases where onset is with E. N and those where it is thou E. N For making such comparison it was necessary to collect series of fairly acute cases without E. N e. cases in which chest X-ray was known to ha been normal within short time before onset of the disease. Unfortunately such cases *constitute* ery small group.

Material and Methods

In the sarcoidosis material from St. Göran Hospital w ha so far found 44 patients without erythema nodosum, and with a normal X-ray within *two* years before detection of the disease. Out of these patients, 19 had had normal chest X-ray within one year before the first positiv X-ray i. e. in these cases the *disease had duration of less* than one year and was thus fairly acute. The remaining 25 patients, with a normal X-ray from one to tw years befor onset, ha been classified as belonging to the subacute type.

The group mentioned consisted of 16 men and 28 women. The majority were between *20 and 40 years of age the youngest patient* was 15 years and the oldest 74 years old. The mean age was 32 years. In all the cases biopsy disclosed histopathological picture compatible with sarcoidosis. Cultures and guinea-pig tests with gastric lavage fluid and lymph-node material were negative for tubercle bac*teria without exception.*

For all 44 patients the follow-up period was not less than one year and in 57 it was continued for at least two years after the first positiv chest X-ray No corticosteroid treat ment was given within the observation period.

Out of the 44 cases 30 had been found on routine examination. A few of these had some slight symptoms of arthralgia or indefinite retrosternal pains. In the rest of the group, 14 patients, X-rays were taken because the patients manifested mor pronounced symptoms fever cough, shortness of breath, and joint or acute eye symptoms. In 7 of the 28 women the thoracic lesions appeared after delivery or gynecologic operation in

TABLE I Present material Pulmonary changes t first positive X-ray

Established duration of disease	No. of cases	Bilateral hilar lymphadenopathy	Paratracheal lymphadenopathy and/or parenchymal lesions
Less than 1 year (acute cases)	19	12 (63.2 %)	7 (36.8 %)
1—2 years (subacute cases)	5	8 (32.0 %)	17 (68 0 %)
Less than 2 years (acute and subacute cases)	44	20 (45.5 %)	24 (54.5 %)

TABLE II Comparison of prognosis of early sarcoidosis without and with erythema nodosum

Type of cases	One-year follow up		Two-year follow up	
	No. of cases	Recovery and normal X ray	No. of cases	Recovery and normal X-ray
Acute non-E.N (duration less than 1 year)	19	11 (57.9 %)	15	12 (80.0 %)
Subacute non-E.N (duration 1—2 years)	25	9 (36.0 %)	22	13 (59.1 %)
Acute and subacute non-E.N (duration less than 2 years)	44	20 (45.5 %)	37	5 (67.6 %)
Erythema nodosum (Lofgren 1953)	111	71 (64 0 %)	111	102 (91.9 %)
Erythema nodosum (Hedvall 1960)	33	17 (51.5 %)	33	25 (76.0 %)

three patients swelling and tenderness of an old scar was the first sign of sarcoidosis.

The type of lesions in the first positive chest X ray is shown in table I Out of the entire material 20 patients (45 5 %) had bilateral hilar lymphadenopathy only and in 24 (54.5 %) there were also paratracheal lymph nodes and/or parenchymal lesions. The table shows that paratracheal lymph nodes and parenchymal involvement were more common in the group of patients with subacute disease than in the group with a shorter and more acute type of illness, 68 0 and 36.8 respectively The difference is statistically probable.

The one-year and two-year prognoses of the material were compared with those of two Swedish E. N series collected by Lofgren (1953) and Hedvall (1960)

Results

With regard to prognosis there was no difference between men and women and consequently the results are presented irrespective of sex. The one year and the two-year prognoses of the present material and of the two Swedish erythema nodosum series from 1953 and 1960 are given in table II In accordance with many other reports, both the E. N -series show a high incidence of recovery at the two-year level (92 and 76) Lofgren s cases show a somewhat better prognosis.

Prognosis is shown for the material as a whole and also for acute and subacute cases separately These last mentioned groups are small, and there is no statistical difference between them. However t is of interest to

note the trend towards a better prognosis in the acute cases (57.9 % and 80.0 % at the one-year and two-year follow-up respectively as against 36.0 % and 59.1 % for the subacute group).

In no instance was there any statistical difference between the cases without erythema nodosum and Hedvall's E. N. cases. The very good prognosis of Löfgren's E. N. series does not apply to the present material as a whole. Of the non-E. N. cases 20 (45.5 %) recovered within one year and 25 (67.6 %) within two years, compared with 64.0 % and 91.9 % respectively in the 1953 E. N. series. However, if the non-E. N. material is cleaned up and we take into consideration only the small but homogeneous group of acute cases, the favourable prognosis is evident (58 % recovered within one year and 80 % within two years). There is no statistical difference between this group, which is the most suitable for comparison, and the two series of E. N. cases.

Consequently, early sarcoidosis seems, on an average, to have the same good prognosis in the cases without erythema nodosum, as has been previously shown for the cases connected with erythema nodosum.

References

1. Hedvall, E. Acta Tuberc. Scand. 39: 249, 1960.
2. James, D. G. Amer. Rev. Resp. Dis. 84: 66, 1961.
3. James, D. G. Brit. Med. J. 1: 853, 1961.
4. Löfgren, S. Acta Med. Scand. 145: 465, 1953.
5. Löfgren, S. and Stavenow, S. Amer. Rev. Resp. Dis. 84: 71, 1961.
6. Scadding, J. G. Proc. Roy. Soc. Med. 49: 799, 1956.
7. Scadding, J. G. Brit. Med. J. 2: 1165, 1961.
8. Sones, M. and Israel, H. L. Amer. J. Med. 29: 84, 1960.
9. Wurm, K. Der Morbus Besnier-Boeck-Schaumann. Ferdinand Enke Verlag, Stuttgart, 1957.

From Hôpital Bichat, Paris, France.

Prognosis of Primary Lympho-Mediastinal Sarcoidosis

J Turiaf

Between 54 % and 90 % of the cases of primary sarcoidosis recovered spontaneously according to different authors. During the last 5 years we have carefully controlled the development of 33 cases of primary sarcoidosis detected at an early stage. The results are as follows.

All the cases that reached the second stage recovered 2 spontaneously and 7 with corticotherapy Four cases became chronic 3 cases of mediastinal adenopathies and one case that developed toward the third stage despite cortisone

The analysis of the spontaneous recovery periods of primary sarcoidosis reveals that very often it occurs precociously 3 cases in less than two months and 3 cases in less than three months. Two of the 3 patients who recovered in less than two months were young adult women. Sarcoidosis occurred simultaneously with erythema nodosum, fever and arthralgia. Bronchial biopsy and Kveim test were positive. In the 4 other patients who recovered in less than three months sarcoidosis was completely latent.

All the patients recovered before the 18th month. Beyond this period adenopathy seems to be permanent.

The principal aim of this short investigation was to study the forms of primary sarcoidosis which are rapidly and spontaneously curable. They should be recognized and differentiated from the adenopathies of some viroses (ornithoses) and infections (streptococcal infections) by means of bronchial biopsy and the Kveim test. These forms of the disease are probably not fully included in the statistics of sarcoidosis in the population.

Table I Prognosis of lympho-mediastinal sarcoidosis

	No. of cases	Percentage
Spontaneous recovery	20	61
Chronic state	3	9
Development toward second stage	9	27
Development toward third stage	1	3
Total	33	100

Table II Prognosis of primary lympho-mediastinal sarcoidosis. Spontaneous recovery periods

	No. of cases
Less than 2 months	3
2 to 3 months	3
4 to 6 months	6
7 to 12 months	7
13 to 16 months	1
Total	20

Dr. Chapman: These cases of Professor Turiaf have the shortest courses of any I have heard of. Professor Turiaf, si l'amour produit le sarcoïde, comme Dr Löfgren dit hier soir en France l'amour ne persiste pas longtemps. But I don't suppose the duration or intensity of love has very much to do with the prognosis of sarcoidosis.

The series of cases presented this morning permits for the first time a collective experience in regard to early sarcoidosis and its prognosis. The evidence seems entirely to confirm Dr Löfgren' observations with respect to primary sarcoidosis in Caucasians. There are too few Negroes included to permit of clear inference. Experience in the United States, as I interpret it, suggests that the prognosis is not nearly so good and that even erythema nodosum in this group is not a favorable sign. Our general impression is strong that sarcoidosis follows a much more severe course in Negro patients and that spontaneous remission is far less frequent in such individuals.

Dr James: We have had two distinctive themes this morning. One on cardio-pulmonary function and the other on the early manifestation of sarcoidosis. Let us learn to combine both by endeavouring to assess the cardio-pulmonary function in very early sarcoidosis in the future. We have seen the beautiful observations of cardio-pulmonary function in early bilateral hilar lymphadenopathy but let us endeavour to assess function when there is even a normal chest X-ray. Assessing cardio-pulmonary function in chronic pulmonary fibrosis seems to be assessing it at late non-specific end-stage similar to that of, say liver failure or renal failure. I should like to see observations on sarcoidosis directed towards the earliest possible phase of the disease because therein may be the key to the most important remaining enigma — namely its aetiology.

Dr Löfgren: The subject treated in this session seems very interesting to me: how does the presence of the hyperergic skin symptom, erythema nodosum, change the prognosis of early sarcoidosis. Some years ago I dealt with a similar problem regarding primary tuberculosis (1). There the answer appears to be quite clear: the symptom, erythema nodosum, implies an unfavourable influence on the prognosis.

This fact is obvious already from general experience. Formerly practically everyone had primary tuberculous infection (was tuberculin positive). In most cases, however, the infection was asymptomatic, and comparatively few persons, with primary tuberculous infection, subsequently contracted a real disease.

On the other hand, primary tuberculosis in connection with erythema nodosum was followed, in a high percentage of cases, by exudative pleurisy or progressive pulmonary or generalized tuberculosis.

Regarding early sarcoidosis, I myself have pointed out the good prognosis for erythema nodosum cases (2) and — as Dr Stavenow mentioned — this statement has been confirmed by other investigators. According to experience of material consisting of primary tuberculosis, I am inclined to believe, however, that early sarcoidosis, without erythema nodosum, might have a still better prognosis than acute sarcoidosis connected with erythema nodosum.

Dr Stavenow pointed out that, principally cases of erythema nodosum are acute in type. When erythema nodosum is absent, it is much more difficult to prove that the sarcoid hilar adenopathy is of an acute type. A normal chest X-ray a short time before the detection of the hilar adenopathy is a requisite condition for such a conclusion. Dr Stavenow has tried to collect material of this kind for comparison with erythema patients.

As a first attempt, the erythema nodosum patients were compared with cases of hilar adenopathy where there was a maximal interval of two years between normal and pathological chest X-rays. Accordingly in some of these cases the disease may have had a duration of nearly 2 years, i.e. it was not acute but subacute. In this comparison the erythema nodosum group had better prognosis.

When the interval in question was reduced to a maximum of one year the number of cases available was, of course, diminished. But the prognosis was then found to be about the same as that for the erythema nodosum cases.

I consider however that even an interval of one year between normal and pathological chest X-ray is too long to make the prognostic comparison relevant. If it were possible to obtain material where the interval was three or four (or six) months, then we really would have a group of comparable, fresh cases; and I should not be surprised if such patients, with really acute sarcoidosis, would have a better average prognosis than that of the erythema nodosum cases.

References

1. Löfgren, S. Le Poumon 1949. 147
2. Löfgren, S. Acta med. Scand. *145*: 465, 1953.

Dr Scadding: I agree with Dr Löfgren about the importance of relating the prognosis of early sarcoidosis to its true duration; but this is relevant principally to theoretical interpretations. In re-

lation to practical problems, the sort of figures which he and I and others among us have published, relating prognosis to the facts available to us when a patient actually comes under our care remain valid they permit us to say that an onset with erythema nodosum indicates a favourable prognosis. If it can be established that an onset with bilateral hilar adenopathy known to have been present for only a short time, is even more favourable, this knowledge will be equally valuable, though in accordance with expectation.

It is striking that the incidence of erythema nodosum in relation to sarcoidosis is widely different from population to population. Here in Scandinavia, it seems to be high, with us in England intermediate, and in the United States low As far as I can gather the same relative incidence holds for erythema nodosum in primary tuberculosis, to judge from the figures for nurses published by Dr Heimbeck from Oslo, in the Prophit report from England, and by Dr Israel and his colleagues from Philadelphia. Of course, this interesting parallelism can be interpreted however you like in relation to aetiology

The Therapy of Sarcoidosis

From St. Göran's Hospital, Stockholm, Sweden.

The Therapy of Sarcoidosis

Nils Svanborg

As a rule, the prognosis of early sarcoidosis is decidedly good, the parenchymal lesions often show spontaneous regression (1, 2).

In a previous report (7) patients were described who, during 1 or 2 years, had severe pulmonary sarcoidosis but subsequently displayed spontaneous regression and afterwards had a good working capacity and fully normal cardiopulmonary function.

The efficacy of corticosteroids in the treatment of sarcoidosis is established. Radiographic clearing is often seen after treatment, but, at the same time, functional improvement is less pronounced (3, 4, 5). Cortisone treatment cannot possibly have any effect on scar tissue already present, but if the patient with progressive pulmonary involvement does not receive treatment, increasing granulomatous lesions could give rise to more severe pulmonary insufficiency. We agree that treatment with steroids is often necessary in hypercalcaemia, ocular, central nervous, myocardial, and progressive pulmonary involvement. In such cases, when treated with conventional doses of cortisone, considerable relief of the symptoms, regression in lung X-ray, increasing lung volumes, etc are often observed. The problem is that, in individual cases, cortisone might possibly still have a fibrous-promoting action. It is remarkable that in numerous cases where clinically good results and functional improvement are reported, the so-called diffusing capacity is still severely impaired (3, 4, 5).

As an example, I should like to describe one of our patients. A 43-years-old plater previously healthy with a normal lung X-ray in 1956. From July 1962 increasing dyspnea. X-ray from March 1963 (fig. 1) disclosed disseminated densities in both lungs. He had shortness of breath at rest, and slight cyanosis of lips. Biopsy from a typical skin scar showed sarcoid changes of fairly fresh appearance. After 4 months' treatment with cortisone (tablets and inhalations) he felt much better and the lung-fields had cleared up (fig. 2).

The functional study before and after 4 months' treatment with cortisone showed that the small lung volumes and (the airflow) the maximum voluntary ventilation (MVV) were normalized, and the working capacity much improved.

Before treatment, he worked 300 kpm/min and after 4 months' treatment with cortisone 600 kpm/min with the same pulse rate of 113 beats/min. The diffusion capacity was remarkably low before treatment and showed little increase after treatment. The alveolo-arterial oxygen gradient was lower after cortisone, possibly as a result of a better relationship between ventilation and perfusion after treatment. The capillary blood volume was normal before treatment.

Impaired diffusion in sarcoidosis might be due to irreversible parenchymal changes, presumably fibrotic in character and possibly more important when localized in the alveolo-capillary area. Nevertheless the possibility cannot be excluded that in some patients cortisone treatment might, for unknown reasons, accelerate healing through fibrosis. Undoubtedly the unusual patient, with acute pulmonary insufficiency from progressive, diffuse, miliary nodulation or broncho-

lation to practical problems, the sort of figures which he and I and others among us have published, relating prognosis to the facts available to us when a patient actually comes under our care remain valid they permit us to say that an onset with erythema nodosum indicates a favourable prognosis. If it can be established that an onset with bilateral hilar adenopathy known to have been present for only a short time, is even more favourable, this knowledge will be equally valuable, though in accordance with expectation.

It is striking that the incidence of erythema nodosum in relation to sarcoidosis is widely different from population to population. Here in Scandinavia, it seems to be high, with us in England intermediate, and in the United States low As far as I can gather the same relative incidence holds for erythema nodosum in primary tuberculosis, to judge from the figures for nurses published by Dr Heimbeck from Oslo, in the Prophit report from England, and by Dr Israel and his colleagues from Philadelphia. Of course, this interesting parallelism can be interpreted however you like in relation to etiology

From the Sarcoidosis Clinic, Jefferson Medical College Hospital, Philadelphia, Pennsylvania.

Steroid Therapy in Sarcoidosis[1]

HAROLD L. ISRAEL

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The actual effectiveness of steroid therapy in sarcoidosis can be established only by controlled studies, and thus far none has been reported. It was the recommendation of the conference in Washington in 1960 that controlled studies be instituted. We have designed such a study and our experience may be helpful to others contemplating such an investigation.

Eligible for admission to the study are patients with active sarcoidosis who have not previously received adrenal steroid therapy and who have neither hypercalcemia nor ocular, myocardial, or neurologic involvement for which steroid therapy is essential. Tablets of prednisone (5 mgm.) and of lactose have been prepared by the manufacturer, Schering, and provided in bottles having consecutive code numbers selected at random. Each bottle contains a three month supply of tablets to be taken three times daily.

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Fig 2 — K.K.A ♂ 43 years. Lung X ray Aug 1963 after 4 months cortisone treatment.

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	Before	After
VC, l	3 0	4.1
RV l	1.3	1 7
$FEV_{1.0}$, l	2.6	3.3
MVV_{free} l/min	74	15?
Compliance l/cm H_2O	0 13	—
Resistance cm H_2O/l/sec.	1.8	1 4
N_2-washout time min	1 0	1.6

	Before	After
Work load kpm/min	300	600
Pulse rate beats/min	113	113
$D_{L_{CO}}$ ml/min/mm Hg	12.9	18.5
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2. Lofgren S. Acta Med. Scand. *145*. 424 465 1953

3 McClement J H., Renzetti, A. D Himmelstein A. and Cournand, A. Amer Rev *Tuberc. 67 1-4 1953.*

4 Riley R L., Riley M C and Hill, H. M *Bull.* Johns Hopk. Hosp. *91 345 1952*

5 Snellie, H. Apthorp C H and Marshall, R. Thorax *16* 87 1961

6. Stone, D. J Schwartz A., F than, J A. and Lovelock, F J Amer J Med. *15*. 468, 1953

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Work load kpm/min	300	600
Pulse rate beats/min	115	113
D_{LCO} ml/min/mm Hg	12.9	18.5
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MVV_{free} l/min	74	132
Compliance l/cm H_2O	0.13	—
Resistance cm H_2O/l/sec.	1.8	1.4
N_2-washout time min.	1.0	1.6

	Before	After
Work load kpm/min	300	600
Pulse rate beats/min	115	115
$D_{L_{CO}}$ ml/min/mm Hg	12.9	18.5
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$(P_A - P_a)_{O_2}$ mm Hg	47	20
$\dot{Q}_{SH}/\dot{Q}_T$	1.3	0.2
V_C, ml	150	45
W_{max}, kpm/min	600	1 000

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From the Sarcoidosis Clinic, Jefferson Medical College Hospital, Philadelphia, [illegible]

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TABLE 1

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RV l
FEV_1
MVV_f
Compl
Resist
N_2-w
Work
Pulse
D_{LC}
D_{M}
$(P_A$
$\dot{Q}$
V_C
W

Tal

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The following studies are made:

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Between March 1962 and July 1963 twenty-three patients with sarcoidosis were considered for admission to the study. One was rejected because of acute uveitis, another because of diabetes mellitus, and a third refused to participate in an experimental investigation.

Of 20 patients enrolled in the study one became pregnant and was discharged, and another was lost to observation. Further losses for various reasons must be anticipated during the ensuing five year interval which must be regarded as a minimum for evaluation of the long term effects of prednisone treatment.

In most instances the clinical response has made obvious whether steroids or the placebo was administered. On the other hand, it has been instructive to encounter some examples of dramatic spontaneous improvement: one young woman with splenomegaly and hypersplenism of several months duration improved remarkably after starting treatment, which proved subsequently to have consisted of lactose tablets!

A long term evaluation of this type does not require that physician and patient be ignorant of the medication given since subjective evaluation of symptoms will be of little importance. The effectiveness of therapy will be measured largely by the results of serial roentgenograms and spirograms. What is essential is that strictly random allocation of medication is practiced so that no bias influences selection.

It is evident that large numbers of patients must be entered in controlled studies in order to obtain sizeable and comparable samples at the end of the five year interval. We have attempted to enlist the cooperation of other investigators in order to swell the size of the groups, but the number of patients enrolled in other clinics has been disappointingly small.

It appears that only a determined and long sustained effort will provide the data necessary to ascertain whether corticosteroid therapy should be used for suppression of clinical manifestations or whether it deserves wider application in sarcoidosis.

Thanks are due Roger W. Cooper, M. D., Medical Research Division, Schering Corporation, Bloomfield, New Jersey for providing prednisone and placebo medication.

From the University Lung Clinic, Renströmska sjukhuset, Göteborg, Sweden.

Long Term Steroid Medication in Advanced Pulmonary Sarcoidosis

Lars Andrén, Erik Berglund and Rolf Malmberg

We wish to present results of treatment with steroids, lasting for one year or more. The results are analyzed as regards changes in clinical state, radiological appearance and pulmonary function.

The material comprises 5 males and 7 females.

7 of the 12 cases seem to be more malignant than the other 5, as indicated by the initial type of observation, see Table I. They showed at the first observation either parenchymal lesions with hilar engagement or parenchymal lesions only. In five cases extrathoracic lesions appeared later on. 11 cases out of the 12 were verified by biopsy.

At the onset of the medication 6 of the patients were in radiological stage II, and the other 6 were in stage II—III or in stage III. In 8 of the 12 cases the known duration of the parenchymal engagement was more than 3 years before treatment.

All the cases treated had the clinical picture of malignant type of disease. All had increasing dyspnoea. 9 had progressive type of disease as indicated by radiological progress. 6 had severe non-productive cough. These 12 cases with prolonged treatment constitute only 14 % of the total hospitalized material of sarcoid patients during this time period. Of the 10 cases studied before first treatment, 8 had abnormal spirometry and the remaining two had abnormal gas exchange alone.

The medication was started while the patients were hospitalized. The drugs were either prednisolone or triamcinolone (Kenacort). The dosages given below are calculated as prednisolone dosages. Initially 20-40-60 mg per day were given for 4—6 weeks. The dosage was then successively lowered to maintenance dose of 5-10-15 mg per day.

Analyses

Of the 12 patients, 7 had an unquestionable clinical improvement during the steroid medication. Of those 7 cases, all were judged to be in stage II except one, who was in stage II—III; four cases initially seemed relatively benign, as indicated by hilar enlargement only but later developed parenchymal lesions. In at least 5 of the cases, there was remarkable improvement, radiologically and subjectively within one month. In one patient there was no sign of improvement within the first 8 months, but later a very marked improvement; this patient apparently had a complete remission with no recurrence in spite of almost 4 years without medication.

One patient stopped the medication on his own. He got a relapse within 6—12 months, and on questioning he then admitted that he had stopped the medication. On renewed therapy he again showed a marked radiological remission. In two other cases the dosage of steroid was temporarily decreased but when the radiological picture showed some relapse the dosage was again increased.

Contrary to these 7 cases, the remaining 5 cases showed no clinical or radiological im-

Between March 1962 and July 1963 twenty-three patients with sarcoidosis were considered for admission to the study. One was rejected because of acute uveitis, another because of diabetes mellitus, and a third refused to participate in an experimental investigation.

Of 20 patients enrolled in the study one became pregnant and was discharged, and another was lost to observation. Further losses for various reasons must be anticipated during the ensuing five year interval which must be regarded as a minimum for evaluation of the long term effects of prednisone treatment.

In most instances the clinical response has made obvious whether steroids or the placebo was administered. On the other hand, it has been instructive to encounter some examples of dramatic spontaneous improvement: one young woman with splenomegaly and hypersplenism of several months' duration improved remarkably after starting treatment, which proved subsequently to have consisted of lactose tablets!

A long term evaluation of this type does not require that physician and patient be ignorant of the medication given since subjective evaluation of symptoms will be of little importance. The effectiveness of therapy will be measured largely by the results of serial roentgenograms and spirograms. What is essential is that strictly random allocation of medication is practiced so that no bias influences selection.

It is evident that large numbers of patients must be entered in controlled studies in order to obtain sizeable and comparable samples at the end of the five year interval. We have attempted to enlist the cooperation of other investigators in order to swell the size of the groups, but the number of patients enrolled in other clinics has been disappointingly small.

It appears that only a determined and long sustained effort will provide the data necessary to ascertain whether corticosteroid therapy should be used for suppression of clinical manifestations or whether it deserves wider application in sarcoidosis.

Thanks are due Roger W. Cooper, M. D., Medical Research Division, Schering Corporation, Bloomfield, New Jersey for providing prednisone and placebo medication.

ing. Before treatment the $P_{A-a}O_2$ was elevated in 5 out of 7 cases studied, with values between 30 and 70 mm. After treatment the $P_{A-a}O_2$ seems to be less pathologic. Also in this group one clinically unimproved patient showed lower $P_{A-a}O_2$ after medication. It is likely that the improvement obtained is mainly due to improved ventilation/perfusion relationship.

On standard exercise tests on bicycle ergometer several of the patients showed moderate or marked improvement in exercise capacity. The improvement appears as lower heart rate and lower respiratory frequency at an identical exercise load. This improvement was closely related to the improvement in gas exchange.

Fig. 3 shows an attempt to correlate the observed changes in pulmonary radiogram with the quantitatively measured changes in vital capacity. The correlation between these two findings seems to be good.

Conclusions

In 12 cases with progressive type of pulmonary sarcoid disease, steroid therapy was given for at least one year. In 7 cases there was significant improvement, usually evident within one month after the onset of therapy. Optimal improvement occurred between one and 24 months of treatment. In one case with initial improvement, there was a temporary marked relapse when the patient stopped the medication.

In those cases on the other hand where no clinical and radiological improvement was obtained, the progressiveness of the disease seems to be totally unaffected by the effect of medication.

The changes in pulmonary radiograms and in ventilatory capacity during the observation and treatment time were well correlated to each other as well as the changes in gas exchange and exercise capacity.

TABLE I Initial observation

Erythema nodosum	1
Hilar enlargement	4
Parenchymal lesion + hilar nodes	1
Parenchymal lesion alone	5
Extrathoracic lesion alone	1
Total	12

provement at all. The functional data on the other hand showed in two of them a slight improvement as shown below. In 4 of the 5 cases, severe fibrosis was present, as shown either by X-ray or by necropsy. The 5th case was judged before the treatment to be in stage II.

In 3 cases medication was given for more than 3 years without any improvement, and *in 2 of these there was no change even 9 and* 17 months after stopping the medication. The progressive course in one of these patients has continued. Two of the patients are dead.

Functional Evaluation

The data on vital capacity in % of predicted value before treatment and after various periods of treatment are shown in Fig. 1. The dotted line at a value of 80% of the normal predicted is considered to be border between normal and pathological values. Before treatment only two patients had a normal vital capacity. After one to six months of treatment, 6 of the patients had a normal vital capacity. After one to five years there were still six patients with normal values. Even in the additional four cases there was a tendency to improvement.

In Fig. 2 are shown gas exchange data. The disturbance in the gas exchange between alveolar gas and the blood is partly due to impaired diffusion but probably mainly due to uneven ventilation/perfusion. It is here measured as the Alveolo-arterial oxygen tension difference ($P_{A-a}O_2$) at room air breath-

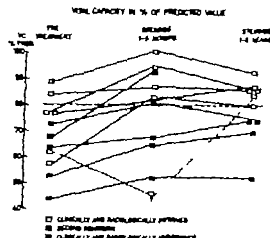


Fig. 1

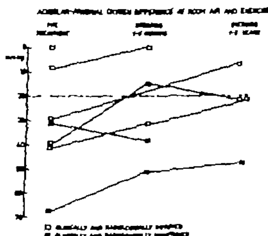


Fig. 2

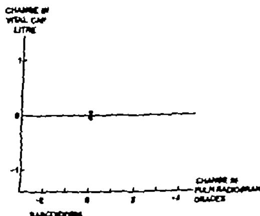


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From the Division of Thoracic Diseases, Dept. of Medicine, The Mount Sinai Hospital, New York.

Chloroquine Therapy in 43 Patients with Intrathoracic and Cutaneous Sarcoidosis

LOUIS E. SILTZBACH and ALVIN S. TEIRSTEIN

During the past two years we have treated 43 patients having sarcoidosis with chloroquine and we can confirm a previous report by Morse et al. (1) that this drug is especially effective suppressing cutaneous sarcoids. The efficacy of chloroquine for the treatment of intrathoracic sarcoidosis is not so clearcut. To our mind, this drug cannot be substituted for corticosteroids in symptomatic progressive pulmonary sarcoidosis or in ocular sarcoidosis.

How can we best judge the value of any new drug in sarcoidosis? Spontaneous resolution in sarcoidosis is a commonplace event, but relapse after spontaneous resolution is quite uncommon. Hence, the frequency rate of "rebound" relapse after halting the drug is a more reliable measure of the drug's suppressive action than is the patient's improvement under therapy.

General Prognosis in Sarcoidosis

Figure 1 presents as background the outcome with and without treatment of 285 patients with biopsy-confirmed intrathoracic sarcoidosis who improved after 4 to 24 months (2). The patients are grouped into three categories according to their initial chest film: group 1, hilar adenopathy alone; group 2, hilar adenopathy and pulmonary mottling; and group 3, pulmonary mottling only. By the end of the first year all three groups experienced substantial radiographic improvement ranging from 69 in patients with hilar adenopathy and mottling to 54 among patients with parenchymal infiltration only. Group I with hilar adenopathy alone, showed the greatest percentage of patients with a completely cleared chest X-ray at the end of the first year 24 while patients with pulmonary mottling showed but 13 % total radiographic clearing.

During the second year improvement was again noted in all three groups. Among patients with hilar adenopathy only, improvement was seen in 75% of whom 34 now showed a normal chest X-ray. Groups 2 and 3 showed lesser degrees of radiographic resolution. These rates of improvement were similar among the corticosteroid treated patients and those not so treated, but only the most seriously ill patients were treated with corticosteroids. One sees therefore that gradual improvement is the trend among average patients with sarcoidosis.

Results of Chloroquine Therapy

We treated 43 patients and they received 250 mg of chloroquine twice a day for periods ranging from four to 17 months, the mean course being eight months. At present, courses are limited to six months to minimize risk of ocular damage. Although minor side effects of the drug, such as anorexia, headache, dizziness, itching and transitory diplopia were experienced by a minority of patients, none of these side effects necessitated stopping treatment. One patient experienced

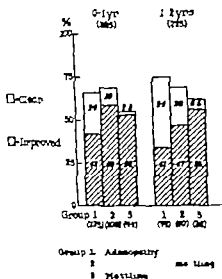


Fig. 1. Status of 285 patients with intrathoracic sarcoidosis after four months to two years according to initial chest film.

clouding of the cornea and blurring of vision after ten months of treatment. The cornea returned to its normal status three weeks after the chloroquine was stopped. No patient suffered retinal damage as manifested by constriction of the visual fields.

For study purposes, patients with all degrees of intrathoracic involvement, both symptomatic and asymptomatic, were treated. Most of the patients had been observed for period of six months or longer prior to chloroquine therapy. A variety of extrathoracic sarcoid lesions were also treated to observe their responsiveness as well. We avoided treating recent ocular lesions of sarcoidosis and lesions posing an immediate threat to vital organ.

Table I shows the results with chloroquine in the group of 43 patients. The total number adds up to more than 43 since some of the patients had combined intrathoracic and cutaneous manifestations and are listed under both headings.

There were 14 patients with hilar adenopathy alone and 11 of these showed significant improvement. In two instances, the chest X-ray returned to normal. In the group of 18 patients with hilar adenopathy plus pulmonary infiltration, 14 showed improvement and two of these also cleared completely. Among 11 patients with mottling only six showed improvement including one with complete clearing. The first radiographic signs of improvement with chloroquine treatment were usually seen in one to three months, with maximum improvement in three to six months. This is a somewhat slower rate of clearing than one sees with corticosteroids.

Table I. Response to Chloroquine — 43 patients with biopsy proved sarcoidosis
The Mount Sinai Hospital (1960–1962)

Site	No. patients	Improved under Rx	Relapsed
1 Intrathoracic			
Hilar adenopathy	14	11	6
Hilar adenopathy and mottling	18	14	3
Mottling only	11	6	2
2 Cutaneous	14	14	9

The proportion of the chloroquine-treated patients showing radiographic improvement is somewhat greater than that shown by the total roster of 285 patients in Table I, especially when one takes into account that the favorable effects of chloroquine took place within three to six months and not in four months to two years. These statistical parallels of rates of improvement are not really as useful in showing the capacity of any drug to suppress sarcoidosis as is the incidence of relapse after cessation of drug.

Relapse after chloroquine was *discontinued* is recorded in the last column of Table I. Almost all of these relapses occurred within two months. Six of 11 patients with hilar adenopathy alone who had improved with chloroquine relapsed, an incidence of recrudescence measurably higher than one ordinarily sees in the course of untreated sar

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During the second year improvement was again noted in all three groups. Among patients with hilar adenopathy only improvement was seen in 75% of whom 34 now showed a normal chest X-ray. Groups 2 and 3 showed lesser degrees of radiographic resolution. These rates of improvement were similar among the corticosteroid treated patients and those not so treated, but only the most seriously ill patients were treated with corticosteroids. One sees therefore that gradual improvement is the trend among average patients with sarcoidosis.

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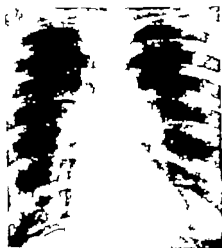


Fig. 4. Same patient at end of second course of chloroquine therapy lasting seven months. The chest X-ray show complete clearing of the pulmonary nodulations.

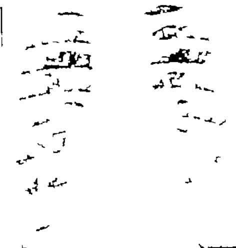


Fig. 5. Same patient showing relapse four months after discontinuation of second course of chloroquine therapy The lungs again show universal nodulation and third course of chloroquine was begun which also caused prompt resolution of the nodulation (not shown)

noted under therapy (Fig. 4). When pulmonary nodules persist for year clearing without treatment is not likely

Four months after discontinuing chloroquine, diffuse pulmonary mottling recurred (Fig. 5) Prompt clearing of the pulmonary lesions again occurred after third course of chloroquine therapy

Relapse after spontaneous clearing of pulmonary infiltrations is quite uncommon in our experience. In this patient relapse was noted after discontinuing drug therapy on three separate occasions, and this, to our mind, demonstrates the suppressive capacity of chloroquine in intrathoracic sarcoidosis.

Response with re-treatment is more easily observed in relapsing cutaneous sarcoidosis as is demonstrated by another patient.

This was 31 year-old Puerto Rican-born woman with hilar node enlargement on chest X-ray and negative reaction to 250 units of tuberculin, who showed classical nodular and lary cutaneous sarcoids in 1957 The cutaneous lesions subsided temporarily with corticosteroid therapy but recurred promptly after the drug was stopped and the lesions spread slowly for the following fiv years. In Figure 6, upper left, are depicted the skin lesions on the back before beginning chloroquine therapy in 1962 Lower left is pre-treatment biopsy showing the classical microscopic picture of cutaneous sarcoidosis. In the right upper frame, five months after chloroquine therapy the skin looks almost entirely clear except for areas of deep pigmentation Below on the right, biopsy of an appropriate area of skin of the back show relatively normal appearing dermis with minimal perivascular nonspecific inflammatory cell infiltration.

In summary it seems to us that chloroquine does possess the capacity to suppress the granulomatous lesions of sarcoidosis. Corticosteroids act more quickly and predictably and are on all accounts the drug of choice for patients with symptomatic, progressive pulmonary involvement as well as for patients with activ ocular lesions, persistent hypercalcemia, hypersplenism and other localizations which threaten organs and even life.

Chloroquine may be considered second

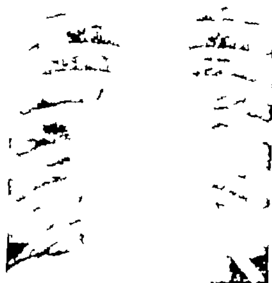


Fig 2. Chest X ray before beginning of first course of chloroquine showing bilateral hilar node enlargement with relatively clear lung fields.

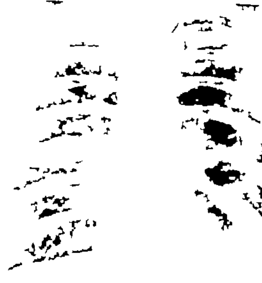


Fig 3 Relapse with universal nodulation of both lung fields with slight prominence of hilar node shadows one year after discontinuing first course of chloroquine. Second course of chloroquine begun.

coidosis. Ten of the 14 patients shown with hilar node enlargement plus pulmonary mottling also improved. Three of these 10 patients showed prompt relapse. Likewise two of six patients with pulmonary mottling only relapsed. This high incidence of prompt relapse occurring as it did two months after halting chloroquine is obviously greater than is usually encountered among untreated patients.

With regard to cutaneous sarcoids, we obtained our most striking results here with chloroquine as Morse et al. did (1) All 14 patients showed some degree of improvement with flattening of lesions and loss of rubor which, at times, left behind flat areas of both increased and decreased pigmentation In many instances the estheti results were extremely pleasing to the patient who might have suffered disfigurement for many years. But relapse after halting therapy was the rule, occurring in nine of 13 patients who completed their course of treatment with improvement in the cutaneous lesions. One patient is still on her first course of therapy making 14 cases in all. Some of these patients had been treated for their cutaneous lesions twice and three times with uniformly gratifying results while the drug was maintained.

Regression of minor localizations of sarcoidosis in the lacrimal and parotid glands as well as in peripheral lymph nodes, paralleled the results seen in intrathoracic and cutaneous lesions.

Illustrative Cases

The following case of intrathoracic sarcoidosis illustrates some of the points made above regarding chloroquine therapy The patient had been observed over a period of three years. A pre-treatment film showed bilateral hilar nod enlargement (Fig 2) While the patient was receiving chloroquine for three months the hilar nodes underwent shrinkage which is not at all unusual in cases of untreated sarcoidosis. Two months after discontinuing chloroquine the hilar nodes enlarged again and, for the first time, pulmonary mottling was noted. These events are also not unusual in the natural history of untreated sarcoidosis. The diffuse nodulation gradually increased over a period of one year to the point where a second course of chloroquine was required (Fig 3) After six months almost complete clearing of the parenchyma was



Fig. 4. Same patient at end of second course of chloroquine therapy lasting seven months. The chest X-ray shows complete clearing of the pulmonary nodulations.

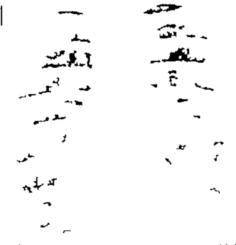


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Fig. 2 Chest X-ray before beginning of first course of chloroquine showing bilateral hilar node enlargement with relatively clear lung fields.

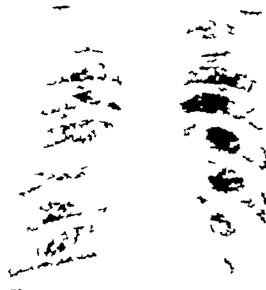


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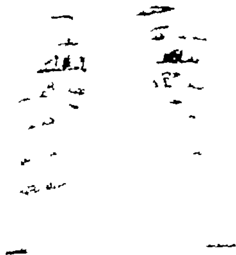


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Dr. James: I started using mepacrine for the treatment of sarcoidosis in 1951 at the Middlesex Hospital because Dr. Francis Page had shown its value in lupus erythematosus. Since that time I have tried chloroquine and hydroxychloroquine, but the overall results have been disappointing. The only true way of evaluating such treatment is by means of a double blind controlled trial. Short of this, the only way is to try to assess the values of these agents in chronic permanent sarcoidosis. It is a waste of time trying to assess its value in acute transient self-limiting sarcoidosis. If the claim for chloroquine is to be substantiated, then it should be able to improve such persistent lesions as lupus pernio and bone cysts. I have never seen these two conditions favourably influenced by the chloroquine group, nor have I seen sarcoid histology influenced in any way.

If you are going to use chloroquine for sarcoidosis, please remember its toxic effects on the eye. It may give rise to reversible corneal deposits and to irreversible retinopathy. These eye complications can be extremely sinister in a disease like sarcoidosis which involves the eye in any case. A physician may unwittingly confuse the chloroquine toxicity with ocular sarcoidosis with tragic results. One quick way to pick up corneal damage due to chloroquine is to ask the patient to look up to an electric light bulb. If he can see a halo round the electric light bulb, then chloroquine should be stopped immediately for this signifies corneal involvement by chloroquine.

Dr. Siltzbach: My studies with chloroquine convince me that it exerts a suppressive action on the lesions of sarcoidosis. As I stated, I think that its action is reflected in the high rate of relapse which occurs after the drug is halted. I cannot remember the ocular damage which uncommonly results from its use. Thus far we have been fortunate, perhaps, because we limit our course of treatment in most instances to six months. But many rheumatologists all over the world are using this agent and their experience will probably settle the matter one way or the other regarding its continued use in sarcoidosis.

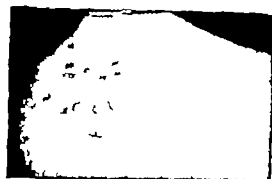
Dr. Israel: I have had one patient who developed retinopathy after prolonged chloroquine treatment. I do not believe the use of this drug is justified in sarcoidosis for a longer interval than 6 months.

Dr. [illegible]: Since many years we have treated cases with chronic parenchymatous sarcoidosis in the lungs in our clinic with long-term steroid therapy. We are quite convinced that it has been of great value for the patients. On the other hand, however, we want to be sure that the condition won't improve by itself. The indications so far have been advanced parenchymatous lesions with progressive dyspnoea for a long time threatening the capacity for work of the patient. I should appreciate very much to hear when the experienced sarcoidosis specialists at this symposium use to start such a treatment. Do you for instance start one year after detection of disseminated lesions in the lung? Do you stress the progressive trend of the disease? Do you find the functional examination, especially repeated examinations, as help in the decision?

Dr. Ba[illegible]: On ne peut pas avoir d'opinion catégorique sur les effets de la Cortisone au cours des sarcoïdoses pulmonaires. Nous insistons seulement sur le fait que certaines lésions infiltratives et densifiantes et des signes radiologiques importants, peuvent être remarquablement influencées. À l'opposé des séquelles de miliaire et de fibrose interstitielle diffuse risquent de ne subir aucune amélioration fonctionnelle malgré des signes radiologiques meilleurs.

Dr. Siltzbach: Like everyone here we promptly treat with corticosteroids patients with ocular and myocardial lesions, persistent hypercalcemia and hypersplenism and those with severe alveolar capillary block. We also treat patients with progressive pulmonary lesions but preferably after a six-months observation period. Perhaps even more important, with long continued maintenance hormonal therapy we are able to render some patients who are totally incapacitated by pulmonary insufficiency fit enough to return to work. We regard this as one of the outstanding accomplishments of corticosteroid therapy in sarcoidosis. Regarding prophylactic isoniazid therapy we employ this for patients who are to be treated with steroids when their tuberculin test is positive. On occasion we give isoniazid even when the tuberculin test is negative if the lung lesions are particularly widespread and progressive. Our incidence of tuberculosis among steroid treated sarcoidosis patients is about 2 [illegible] the same as it is among nontreated patients.

Dr. Scadding: Once again I find myself in complete agreement with Harold Israel. Apart from certain categorical indications, such as some sorts of ocular involvement and hypercalcaemia, the only reason for the use of corticosteroids is the presence of symptoms severe enough to warrant relief by what may prove to be indefinitely prolonged treatment with hormones having potentially dangerous side-effects.



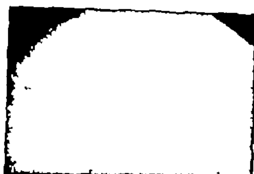






Fig 6. *Upper left*, pre-treatment ppearance of nodular waxy cutaneous sarcoids spreading slowly over a five-year period. *Lower left*, classical epitheliod cell tubercles in the dermis showing slight encapsulation. *Upper right*, five months after chloroquine therapy only depigmented areas of the skin are now visible. *Lower right* no evidence of granulomatous involvement of these areas. In the mid-corium some perivascular infiltration with nonspecific inflammatory cells are seen.

drug in the treatment of sarcoidosis. Side effects occur less frequently than with corticosteroids. Chloroquine s chief value lies in its capacity to suppress cutaneous lesions. It appears useful as well in the treatment of persistent sarcoidal lesions localized to non vital organs such as the salivary and lacrimal glands and peripheral lymph nodes when these are troublesome. Chloroquine treatment is also orth a trial in patients with mild or no respiratory symptoms who nevertheless show slowly progressive pulmonary lesions radiographically Because of the occasionally serious ocular complications of chloroquine therapy periodic examination for corneal and retinal changes are mandatory (3) To reduce the risk of irreversible damage to the eyes, courses of chloroquine treatment are best limited to a six months period w th several months elapsing between successive courses.

References

1 Morse, S. I Cohn, Z. A., Hirsch, J G. and Schaedler, R. W The Treatment of Sarcoidosis w th Chloroquine. Am J Med. 30: 779—784 1961

2. Siltzbach, L. E. and Berger, H W Prognosis in Sarcoidosis. A Study of 311 Biopsy-Confirmed Cases. To be published.

3. Arden G B., Friedmann, A. and Kolb, H Anticipation of Chloroquine Retinopathy The Lancet 1 1164 (June 2) 1962.

This study was supported by Grant AI-02272 from th N tional Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

Dr James I started using mepacrine for the treatment of sarcoidosis in 1951 at the Middlesex Hospital because Dr Francis Page had shown its value in lupus erythematosus. Since that time I have tried chloroquine and hydroxychloroquine, but the overall results have been disappointing. The only true way of evaluating such treatment is by means of a double blind controlled trial. Short of this, the only way is to try to assess the values of these agents in chronic persistent sarcoidosis. It is a waste of time trying to assess its value in acute transient self-limiting sarcoidosis. If the claim for chloroquine is to be substantiated, then it should be able to improve such persistent lesions as lupus pernio and bone cysts. I have never seen these two conditions favourably influenced by the chloroquine group, nor have I seen sarcoid histology influenced in any way.

If you are going to use chloroquine for sarcoidosis, please remember its toxic effects on the eye. It may give rise to reversible corneal deposits and to irreversible retinopathy. These eye complications can be extremely sinister in a disease like sarcoidosis which involves the eye in any case. A physician may unwittingly confuse the chloroquine toxicity with ocular sarcoidosis with tragic results. One quick way to pick up corneal damage due to chloroquine is to ask the patient to look up to an electric light bulb. If he can see a halo round the electric light bulb, then chloroquine should be stopped immediately for this signifies corneal involvement by chloroquine.

Dr Siltzbach My studies with chloroquine convince me that it exerts a suppressive action on the lesions of sarcoidosis. As I stated, I think that its action is reflected in the high rate of relapse which occurs after the drug is halted. I cannot minimize the ocular damage which uncommonly results from its use. Thus far we have been fortunate, perhaps, because we limit our course of treatment in most instances to six months. But many rheumatologists all over the world are using this agent and their experience will probably settle the matter one way or the other regarding its continued use in sarcoidosis.

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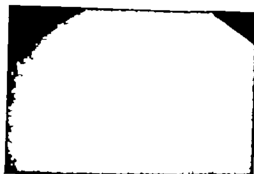
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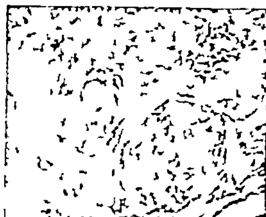
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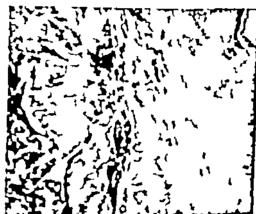


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